ascertain the relative importance of a number of variables simultaneously.

Theoretically, the present analyses might be extended further and one might examine these same relationships separately within an extensive number of subpopulations in order to take into account any interaction effects, most notably those variables which are shown in the tables to have sign reversals between the zero-order relationship and the beta in the final multiple regression equation. Such interactions, when present in the additive model that we have employed, will cause us to underestimate the relationships between predictor variables and outcome measures. Again, such "errors" will result in a more conservative estimate of sex bias. For example, in the present case, since differences in the type and magnitude of sex differentials between different types of institutions and different fields may be expected, the analyses might be performed separately by type of institution and by field or department. However, the present analyses, which combine fields and types of institutions, tend to underestimate the true magnitude of obtained differences. See, for example, *Opportunities for Women in Higher Education*, a report and recommendations by the Carnegie Commission on Higher Education (McGraw-Hill, New York, 1973), sec. 7 and append. C. See also (*6*). Furthermore, this is a replication study of an earlier one based on the status of academic women and men in 1968–69 (*5*) and we follow the same model in order to get an actual comparison over time. Future analyses will involve an examination by field and by type of institution, and will incorporate further study of other interaction effects shown in the present tables.

12. N. M. Gordon, T. E. Morton, I. C. Braden, *Am. Econ. Rev.* **64**, 419 (1974).
13. H. S. Astin, *The Woman Doctorate in America* (Russell Sage Foundation, New York, 1969); A. E. Bayer, *College and University Faculty: A Statistical Description*, ACE Research Report 5:5 (American Council on Education, Washington, D.C., 1971); D. Chubin, *Am. Sociol.* **9**, 83 (1974); A. S. Rossi and A. Calderwood, Eds., *Academic Women on the Move* (Russell Sage Foundation, New York, 1973).
14. R. A. Lester, *Antibias Regulation of Universities: Faculty Problems and Their Solution* (McGraw-Hill, New York, 1974).
15. The analytical method is limited in its capacity to ascertain relative weights and the differential impact of a large set of independent variables. In stepwise regression, minor differences in correlations can produce different orders of entry. Some of the independent variables are also somewhat redundant with others, so that which enters in precedence to another may be due in part to chance. Finally, some variables may have greater variance for one sex than the other and so be more likely to enter the equation. However, between-sex comparison of multiple correlations, and the application of the full equation to the opposite sex, are the primary objectives of the present analyses and are appropriate to the present method. See L. G. Humphreys, *Am. Educ. Res. J.*, in press.
16. Others have recently reported findings similar to ours with respect to increasing sex differentials with increasing experience. See, for example, G. E. Johnson and F. P. Stafford, *Am. Econ. Rev.* **64**, 888 (1974), and J. A. Centra, *Women, Men and the Doctorate* (Educational Testing Service, Princeton, N.J., 1974). Inasmuch as adequate longitudinal data are not employed in these studies, however, it cannot be ascertained whether this increasing disparity is inherent in the career progressions of academic personnel, as these researchers suggest, or whether equity is just now being achieved for newer recruits and will be sustained during their careers.
17. This slight change in proportion represents the following estimated numerical change: In 1968–69 there were approximately 517,000 faculty members in higher education, of whom 99,000 were women. In 1972–73 the total was approximately 620,000, of whom 124,000 were women. Therefore the average annual rate of increase of women on faculties was 6.3 percent during the period; for men it was 4.7 percent, the latter percentage being derived, however, from a substantially larger base-year figure.
18. A. M. Cartter, "The supply and demand for new college and university faculties," in preparation.
19. Supported by a subcontract from the American Council on Education to Florida State University, under grant SSH72-03432 A02 (formerly GI-34394) from the Research Applied to National Needs (RANN) Program of the National Science Foundation. The results and interpretations herein do not necessarily reflect the position of these agencies and are solely the responsibility of the authors.

RNA Processing and RNA Tumor Virus Origin and Evolution

RNA tumor virus genomes may originate from cell DNA via an alternative mode of RNA processing called paraprocessing.

David Gillespie and Robert C. Gallo

Viruses are generally considered to have evolved from cell nucleic acids, but many viruses bear no genetic relationship to the cells they infect. This appears to be true for virulent bacteriophages (*1*), and is assumed to be the case with most plant and animal viruses. Viruses of this type probably could not form a persistent association with host cell DNA. Some bacteriophages have the capacity of interacting with host cell DNA and possess some genetic information similar to sequences in host DNA (*1*). Recombination between the phage genome and host cell DNA results in the physical insertion of phage genes into the host genome (*2*). In this lysogenic state (*3*), genes are expressed at low levels and duplicated indefinitely along with the host cell DNA. Occasionally the inserted bacteriophage information is excised from the host genome (*2*). Fragments of adjacent host cell information can be excised along with the phage genome, and if this occurs the resulting phage genome contains both viral and cellular components (*2–4*).

The cell-like components found in the genomes of most temperate bacterial phages appear to arise from an interaction of the established viral genome with host cell information. If bacteriophages did originate from cellular genetic information, the genetic elements of the phage itself and those of its host have since diverged to the extent that they no longer share most nucleotide sequences. The situation with the RNA-containing animal tumor viruses is different. Evidence from molecular hybridization experiments suggests that the RNA genomes of these viruses have nucleotide sequences that are similar to sequences found in DNA of normal cells. This indicates that RNA tumor viruses (*5*) were relatively recently generated from host cell information. The point of origin of most RNA tumor viruses can probably be measured as having occurred within the last tens of millions of years (*6, 7*). Biological experiments indicate that the viruses are still being generated from cells.

Results from molecular hybridization, from physical analyses of RNA from RNA tumor viruses, and from biochemical analyses of infected animal cells has suggested to us that events in RNA processing determine whether a particular cellular RNA transcript can acquire the potential to become the genome of an RNA tumor virus or whether it will become a messenger RNA (mRNA) molecule. In this article we propose that the type of RNA processing ("paraprocessing") that leads to the formation of an RNA tumor virus genome involves relatively little RNA cleavage in the nucleus. We further suggest that paraprocessing is a form of RNA processing used normally for the expression of particular genes during early stages of differentiation but not normally in mature adult cells.

The results leading to these ideas rely in part on an estimation of the genetic rela-

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tion between RNA tumor viruses and their uninfected host cells from measurements of the formation of complexes between RNA purified from RNA tumor viruses and DNA extracted from the host. The experiments discussed below have been designed to detect regions of similarity between the viral RNA and cell DNA, in addition to regions of identity between them. Therefore the conditions of hybrid formation and detection are important. Regions of *identity* are indicated when RNA and DNA form a hybrid structure stabilized entirely by Watson-Crick hydrogen bonds (a "perfect" hybrid). When mammalian cell DNA is used, such a structure is usually achieved when the formation of imperfect RNA·DNA is selected against, namely, at high temperature and low ionic strength (for example, at 65° to 70°C in 0.2 molar sodium ion), and is indicated by the formation of a hybrid structure with a high thermal stability—for example, 86.5°C in 0.15*M* Na^+ for a hybrid composed of 50 percent of guanine, plus cytosine (*8*).

Regions of genetic similarity between virus RNA and cell DNA are indicated by the formation of hybrid structures stabilized by Watson-Crick base pairs interspersed with other types of base pairs or with unpaired bases ("mismatched" or "imperfect" hybrids). A hybrid containing mismatches is less stable than a perfect hybrid, and thus has a lower thermal stability (t_m) (*9*). The formation of mismatched complexes is encouraged at low temperatures and high ionic strength (for example, at 60°C in 0.5*M* Na^+) and is indicated by the formation of structures with low thermal stability. Studies designed to detect both regions of similarity and identity between RNA of tumor viruses and DNA of host cells therefore require hybridization conditions that are not stringent. However, when mismatched complexes are sought, one must be concerned with the possibility that partially complementary sequences are present because of chance. If such is the case with the hybridization of viral RNA to cell DNA, then RNA isolated from several different RNA tumor viruses and hybridized to DNA from several different species would probably yield no sensible pattern. The fact that a pattern is obtained, as described below, is indicative of the specificity of the hybridization reaction.

In the discussion that follows we have tried to delineate—so far as is known—the relation between the genetic information of RNA tumor viruses and that of particular cell genes, and we suggest a specific mechanism (RNA paraprocessing) whereby a normal cell may give rise to an RNA tumor virus genome.

Class 1 and Class 2 RNA Tumor Viruses

Molecular hybridization between the genomic RNA of RNA tumor viruses and a vast excess (by weight) of DNA from uninfected progenitor cells provides a measure of that portion of the viral RNA for which similar sequences exist in the cell DNA. When this type of hybridization reaction is carried as close to kinetic completion as possible, the results permit RNA tumor viruses to be classified into two groups (Table 1) (*7, 10*). The group we define as class 1 consists of viruses with an RNA genome closely related to that of its progenitor cell. At least 75 percent of the RNA from class 1 viruses anneals to progenitor cell DNA, giving rise to a hybrid structure of high thermal stability (that is, having little mismatching). The t_m's of the hybrid structures, as analyzed by resistance to ribonuclease, are comparable to the t_m of 86.5°C for a ribosomal RNA and cell DNA hybrid formed and analyzed under optimum conditions (*8*). Assay by the hydroxyapatite technique also indicated a high t_m for hybrids of class 1 virus RNA and cell DNA (*11*), but we know of no good reference standard to use as a comparison. The class 1 viruses include RAV_0 chicken virus (*11*); strain RD114 cat virus (*11*) (Table 1); the "endogenous" guinea pig virus (*12*); MMTV, a mouse type B virus (Table 1) (*10*); and probably the baboon endogenous type C virus (*13*) and the xenotropic mouse viruses.

The group we call class 2 consists of viruses with an RNA genome that interacts to only a limited extent (15 to 30 percent) with progenitor cell DNA (Table 1). Moreover, the thermal stability of the hybrid complexes, as measured by resistance to ribonuclease, is low relative to that of hybrid structures obtained with RNA from class 1 viruses. This indicates that class 2 viruses are more distantly related to cells than are class 1 viruses. The class 2 viruses include [see (*7, 10*) or Table 1 when references are not given] AMV (*14*) and Rous chicken viruses (*15*); FeLV cat virus; mouse leukemia and sarcoma-leukemia viruses, strains

Table 1. Hybridization of RNA tumor virus RNA to DNA from uninfected natural host cells. Results are presented as percentages of the RNA hybridized. Thermal stability was measured at 0.15*M* Na^+ at 10^3 C_0t. The conditions of hybrid formation and detection are outlined in the legend of Fig. 1. Hybridization of RNA to repeated sequences in cell DNA was detected by annealing low amounts of DNA (50 μg) to 0.5 ng of viral RNA and terminating the reaction at a C_0t of 10^3. Under this condition, no annealing to infrequent DNA sequences was detected (*10*). Hybridization of RNA to I plus R sequences of DNA was detected by annealing large amounts of DNA (500, 1000, or 2000 μg) to 0.5 ng of viral RNA and terminating the reaction at a C_0t of 25×10^3. In the high C_0t hybridizations the amount of hybridization was the same with all three amounts of DNA tested; only the values with 1000 μg are presented.

Source of virus RNA	Source of cell DNA*	Hybridization results (percent RNA hybridized)		
		10^3 C_0t	t_m (°C)	25×10^3 C_0t
	Class 1 viruses			
Mouse mammary tumor virus, strain DW	Mouse	45	83	100
Cat virus, strain RD114	Cat	40	86	>70†
	Cat	45†		>70†
Chicken virus, strain RAV_0	Chicken	~30†		>70†
	Class 2 viruses			
Mouse leukemia virus, strain Rauscher	Mouse	19	78	20
Mouse sarcoma virus, strain Moloney	Mouse	12	80	17
Mouse-rat sarcoma virus, strain Kirsten	Mouse	3	73	5
	Rat	19	78	20
Cat sarcoma virus, strain Gardner	Cat	17	80	18
Cat leukemia virus, strain Rickard	Cat	18	79	16
Chicken leukemia virus, strain AMV	Chicken	10†		40
Chicken sarcoma virus, strain Rous	Chicken	10†		30
Simian sarcoma virus	Woolly monkey	0–10		0–10

*DNA was prepared from spleen, liver, skeletal muscle, and thymus of mice, rats, and cats; embryos of chickens; and liver of woolly monkeys. No significant differences were found (the variation was 5 to 10 percent of the value listed) when DNA from different tissues was used. No differences were seen when DNA was isolated from livers or spleens of different strains of mice (C57, BALB/c, NIH Swiss) or different domesticated cats. † Values from (*23, 26, 27*).

Rauscher and Moloney; a sarcoma-leukemia virus of both mouse and rat history, strain Kirsten; and a sarcoma-leukemia virus isolated from a woolly monkey; the simian sarcoma virus; and probably a leukemia virus isolated from a gibbon ape, the gibbon ape leukemia virus.

Information concerning the number of copies of viral-related sequences in a cell can also be obtained. The DNA of animals contains two types of sequences, namely, "unique" and "repeated" sequences. Unique sequences are represented only once in a haploid genome, whereas repeated sequences are found two or more times (*16*). Repeated sequences can in turn be divided into those present as several identical copies or those present as a family of similar sequences. In practice, the exact number of copies of a gene is difficult to measure; accordingly we use the term "infrequent" sequences, rather than the term "unique" sequences. Whether those sequences in cell DNA that hybridize to viral RNA are repeated or are infrequent can be assessed by measuring the rate of hybridization of viral RNA to an excess of cell DNA (Fig. 1) and expressing the results as a function of time multiplied by the initial DNA concentration, that is, the C_0t (*16*). The portion of the curve that rises first (at low C_0t) displays hybridization of the RNA to repeated DNA sequences; the portion of the curve that rises last (at high C_0t) displays hybridization to infrequent DNA sequences. The magnitude of the rise indicates that fraction of the RNA complementary or partially complementary to a given type of DNA sequence.

Hybridization of RNA from class 1 viruses to DNA from uninfected progenitor cells results in a bimodal kinetic curve (Fig. 1). Approximately 40 percent of the RNA anneals to repeated DNA sequences of the cell ($C_0t < 10^3$), while the remainder of the hybridized RNA (60 percent with RNA from MMTV, 30 to 50 percent in the case of RNA from RD114) anneals to infrequent DNA sequences ($C_0t > 10^3$). A similar result has been obtained by hybridizing RNA from a class 1 guinea pig virus to DNA from normal guinea pigs (*12*). One class 1 virus is difficult to assess. The endogenous chicken virus RAV_0 contains RNA of which some 30 percent anneals to DNA at C_0t below 500, but two distinct slopes have not been demonstrated (*11*).

RNA from RLV was selected to illustrate the hybridization behavior of class 2 virus RNA. Hybridization of RNA from this virus to DNA from uninfected progenitor cells results in a unimodal kinetic curve wherein all of the annealing occurs with the repeated DNA fraction (Fig. 1). This pattern was seen with RNA of all class 2 mammalian RNA tumor viruses;

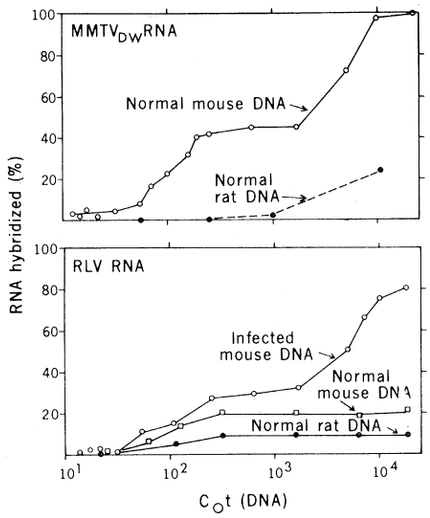


Fig. 1. Kinetics of hybridization of RNA from class 1 and class 2 viruses to DNA from cells. [^{3}H]RNA (0.5 ng; 200 count/min) and DNA (1.0 mg) were mixed in replicate in 0.25 ml of 0.4*M* phosphate buffer and sealed in capillary tubes; the reaction mixtures were then boiled and held at 60°C. At intervals samples were removed, diluted in 0.3*M* NaCl plus 0.03*M* sodium citrate, and incubated with ribonuclease A (20 μg/ml) for 2 hours at 37°C, and precipitated with 10 percent trichloroacetic acid. $MMTV_{DW}$ is the Dmochowski-Williams strain of mouse mammary tumor virus; RLV_R is the strain Rauscher mouse leukemia virus. [Adapted from (*10*)]

however RNA from the class 2 chicken virus RSV anneals primarily to infrequent sequences in chicken DNA (*15*). The apparent difference between RNA of the class 2 chicken virus and that of the class 2 mammalian viruses may be due to the different conditions for hybrid formation. RNA from RSV was annealed to chicken cell DNA at 67°C, a condition that may prevent cross-hybridization among related molecules of a repeated DNA family. The temperature of hybridization of RNA from class 2 mammalian RNA viruses to host DNA (60°C) is that known to permit this cross-hybridization.

The validity of these hybridization experiments can be challenged on the grounds that the viral RNA is heavily contaminated by trapped cellular (nonviral) RNA. Ikawa *et al.* detected one globin mRNA molecule per thousand virus genomes in viruses recovered from hemoglobin-producing cells (*17*). In the case of RNA from the class 1 viruses virtually all of the RNA can be converted to a hybrid structure with DNA from normal cells. In our opinion, contamination to this extent by cell RNA is not probable when viral RNA is isolated as a 50*S* to 70*S* aggregate from an extracellular particle. The RNA preparations used here from MMTV and RD114 were shown by East *et al.* (*18*) to be converted upon heat treatment to "subunits" of discrete size, and these subunits contain polyadenylate [poly(A)], as judged by their ability to bind immobilized polyuridylate [poly(U)]. In the case of RNA from class 2 viruses, no sequences exactly complementary to DNA from normal cells is found, and most of the RNA hybridizes only to DNA isolated from cells producing the virus, not to DNA from normal cells (for example, Fig. 1). The fraction that does form a complex with DNA from normal cells does not behave like a contaminating species of cytoplasmic RNA in that it anneals only to repeated DNA sequences and forms a complex of low thermal stability.

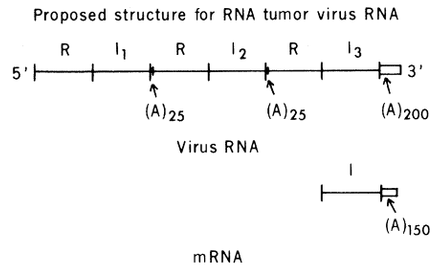
In general, it appears that class 1 viruses are closely related to their hosts, while class 2 viruses are not. The case for the cellular origin of class 1 viruses is strong, especially when it is remembered that five of the six were induced or obtained from apparently normal cells [RD114 or its Crandell virus equivalent (*19*), the guinea pig virus (*12*), RAV_0 (*20*), the baboon endogenous virus (*13*), and the xenotropic mouse virus]. The sixth, MMTV, can apparently be vertically transmitted, from parent to progeny (*21*). Molecular hybridization results are incomplete with the baboon and xenotropic mouse viruses. The case for the cellular origin of the class 2 viruses can be made only indirectly. We have hybridized DNA copies of RNA of different class 2 leukemia viruses (synthesized in disrupted virions by reverse transcriptase) to 60*S* to 70*S* RNA from several RNA leukemia viruses (*6*). The DNA copies always annealed best to 60*S* to 70*S* RNA isolated from the same virus and less to RNA from other viruses. When the data were assembled from selected leukemia or sarcoma-leukemia viruses from birds, cats, mice, and primates, the genetic relationship indicated in Table 2 was found. The mammalian class 2 RNA leukemia viruses are related to one another, and the relatedness pattern is the same as the relatedness pattern of their normal hosts, measured phylogenetically with anatomical or molecular markers. The phylogenetic relatedness pattern was not detected with sarcoma or mammary adenocarcinoma viruses (*6*). The relatedness among mammalian RNA leukemia viruses can also be seen when certain proteins from different viruses are compared immunologically; for example, the relatedness pattern also describes the relatedness among the virus reverse transcriptases (*22*) isolated from the virus cores. This relatedness pattern suggests that the class 2 viruses, like the class 1 viruses, originated from their hosts and evolved away to some extent, or that they originated elsewhere and become cell-like through interactions with cell genomes

(*6*). Since we feel that class 1 viruses do originate from host information and since there is a marked similarity between the structure of class 1 and class 2 viruses, the structure of their RNA, and the structure of their proteins, we accept the cellular origin of both classes of virus as a working model (*23*).

The cell DNA sequences capable of originating RNA tumor virus information have previously been called "virogenes" (*24*). However, we believe that these genes have required functions during normal cellular development and that their ability to create class 1 RNA tumor viruses is incidental; hence, the term virogene is too restrictive and in our context not precise. In this article we use the neutral term "class 1 genes" to describe these host cell DNA sequences.

Paraprocessing of Class 1 Virus Genomes

The expression of genes that code for proteins requires the synthesis of an RNA transcript, the "processing" of that transcript [phosphodiester bond cleavage and poly(adenylation)], the transport of it from the nucleus to the cytoplasm, and the utilization of the RNA (mRNA) as a template for protein synthesis (*25*). We propose that an RNA tumor virus genome is created when an uninfected progenitor cell uses an alternative mechanism to process an RNA transcript copied from a class 1 gene. The alternative RNA processing mechanism is called here "paraprocessing." That cell and its descendants can maintain the presence of the paraprocessed RNA by continued expression of the class 1 gene, but autoreplication of the RNA requires other activities, among them those of the reverse transcriptase machinery. The evidence discussed below is used to explain the origin of the initial molecule of class 1 virus



R = RNA sequences which anneal to repeated sequences in cell DNA

I = RNA sequences which anneal to infrequent sequences in cell DNA ($I_1 \neq I_2 \neq I_3$)

$(A)_{25}$, $(A)_{150}$, $(A)_{200}$ = oligoadenosine or polyadenosine tracts 25, 150, or 200 adenosine residues in length

Fig. 2. Proposed arrangement of nucleotide sequences in polynucleotides of RNA tumor viruses: poly(A) or $(A)_{200}$, tracts of polyadenylic acid 200 residues in length; $(A)_{25}$, tracts of oligoadenylic acid 25 residues in length; RNA sequences which hybridize to repeated sequences of cell DNA are represented by R and are defined by their behavior in hybridization reactions with cell DNA. Different R sequences in the same virus molecule may, but need not, be identical. Infrequent RNA sequences (I) hybridize to infrequent sequences in DNA and may occur only once per genome.

RNA, but it applies as well to the formation of RNA of established class 1 and class 2 viruses in infected cells.

The basis for our proposal that RNA tumor virus genomes are RNA's which have escaped the usual mode of RNA processing comes from an evaluation of the physical properties of RNA with a high molecular weight and its components as isolated from extracellular particles of class 1 or class 2 RNA tumor viruses (Fig. 2). Though the high-molecular-weight RNA from these particles is at one time cytoplasmic, in many respects it structurally resembles RNA isolated from the nucleus of normal cells rather than RNA isolated from their cytoplasm. (i) Sedimentation velocity studies show that the polynucleotide chains of high-molecular-weight RNA (30*S* to 40*S*) from tumor viruses are larger than the average size of mRNA from the cytoplasm of differentiated cells and more nearly the size of RNA from cell nuclei (*26*). In one study, 35*S* RNA could be detected in the cytoplasm of cells infected by and producing an RNA tumor virus while RNA approaching this size could not be found in the cytoplasm of control cells (*27*). Some workers (*28*) observe that nuclear RNA from normal cells is heterogeneous in size and can be of extremely high molecular weight (> 70*S*), but others indicate an aggregate structure composed of several 30*S* to 40*S* polynucleotide chains (*29*). (ii) An aggregate structure for cell nuclear RNA if valid would constitute another similarity between this RNA and virus RNA of high molecular weight.

In any event, the association of several polynucleotide chains in the tumor virus genome differs from the suspected single polynucleotide configuration of cell mRNA. (iii) Tumor virus RNA contains poly(A) tracts approximately 200 residues in length (*30*), a length comparable to that of poly(A) regions of nuclear RNA (*31*) but longer than that of poly(A) segments in cytoplasmic mRNA (*32*). Cellular mRNA undergoes a shortening of poly(A) tracts during its formation from nuclear transcripts (*33*), an event that may not be part of the processing of tumor virus RNA. (iv) As stated above, much of the RNA of class 1 and class 2 tumor viruses is potentially coded by (hybridizes to) repeated sequences in DNA of normal or virus infected cells, respectively (Fig. 1 and Table 1). This, too, is not a feature of poly(A)-containing cytoplasmic cell RNA, but appears to be a characteristic of nuclear RNA.

We believe that the hybridization of

Table 2. Genetic relatedness among class 2 RNA tumor viruses. High-molecular-weight RNA was isolated from isopycnically purified RNA tumor viruses and covalently attached to phosphocellulose filter disks (*61*). This immobilized RNA was hybridized to DNA synthesized by the same set of viruses. Values (percentage of DNA hybridized) are normalized to the homologous cross (50 to 100 percent hybridization). Several preparations of RNA and DNA were used. Hybrids were also analyzed by cesium chloride centrifugation and nuclease digestion (*6, 7, 57*). Virus abbreviations are explained in (*5*).

Cell type that served as source for virus	Virus source of RNA	Source of DNA copies of viral RNA					
		SSV (NC37)	KiMSV (NRK)	MuLV (Gross type)	RLV	FeLV	AMV
Woolly monkey fibrosarcoma (grown in rat or human cells)	SSV (NRK) or (NC37)	90 100	82*	10	8	2 to 5	0
Rat sarcoma (grown in rat cells)	KiMSV (NRK)	85	100	100	10	10	0
Mouse plasma (grown in mouse cells)	MuLV (Gross type)	6 to 10	47	100	10	14	0
Mouse plasma (grown in mouse cells)	RLV	8	14	8	100	10	0
Mouse mammary tumor (produced by tumor cell)	MMTV†	0	0	0	0	0	0
Cat plasma (grown in cat cells)	FeLV	1 to 3	4	6	6	100	0
Chicken plasma (grown in chicken cells)	AMV	0	0	0	0	0	100

*To RNA from SSV (NRK). ‡This RNA hybridized with 100 percent of DNA synthesized by MMTV; the DNA synthesized by MMTV did not hybridize to any other RNA preparation.

some 50 percent of the RNA of class 1 viruses to repeated DNA sequences in cells yields information on the nucleotide sequence arrangement in the virus RNA (Fig. 2). As stated above the repeated DNA fraction can be divided into "families" of sequences whose members bear more similarity to each other than to other families (*16*). It has been proposed that DNA families arise during speciation from the multiplication of a single-copy sequence or of a particular member of an existing family (*34*). In flies, cows, mice, and humans, repeated DNA sequences range from 200 to several thousand nucleotides and are usually located next to an infrequent sequence roughly 750 to 1000 nucleotides in length (*35*). Repeated sequences seem to be most prevalent in higher metazoans. It is assumed that some or all of the infrequent (single-copy) sequences code for proteins while many repeated families of sequences serve an unknown, possibly regulatory, function. Finally, infrequent sequences may terminate in a run of thymidine (T) residues 25 nucleotides in length (*36*). By analogy, the structure in Fig. 2 may describe the sequence arrangement of elements contained in polynucleotides of class 1 tumor viruses. These 30*S* to 40*S* molecules contain four types of nucleotide sequences: (i) those which hybridize to repeated DNA (R sequences); (ii) those which hybridize to infrequent or single-copy DNA (I sequences); (iii) a terminal $(A)_{200}$ sequence [poly(A)] (*30, 37*); and (iv) internal $(A)_{25}$ sequences. By analogy with the arrangement of R and I sequences in genomic cell DNA, we propose that the R and I sequences of RNA tumor viruses alternate. It is important to note that the proposed R and I elements in tumor virus RNA are defined by their hybridization properties to cell DNA. Thus, while R sequences hybridize to a family of related sequences in host DNA, the three to five R elements of a given virus RNA molecule can be different from each other. Since at least some of the I DNA sequences of metazoans codes for mRNA, and since the structure of Fig. 2 contains several I elements, this representation of the RNA is probably polycistronic. If structural genes terminate in $(T)_{25}$ tracts, it follows that RNA tumor virus RNA will contain internal $(A)_{25}$ tracts. The presence in cell nuclear RNA of internal $(A)_{25}$ runs and alternating sequences coded by R and I DNA sequences is implicit in this model and has been well described (*38*).

A reasonable explanation for the observations referred to above is that the tumor virus RNA fails to undergo the type of RNA processing that, in a differentiated cell, cleaves portions of nuclear RNA. Instead, the RNA transcript is processed in another mode, the paraprocessing mode. We envision paraprocessing as an alternative mode of RNA processing usually reserved for the expression of particular genes in undifferentiated cells. In spite of the RNA processing alteration in the differentiated cell, the paraprocessed RNA is transported from the nucleus to the cytoplasm. The initial trigger ("H" change) leading to the formation of a paraprocessed RNA transcript of a class 1 gene is not necessarily a change inducing new transcription of class 1 genes. This hypothesis appears to be supported since some normal adult cells contain in their cytoplasm RNA that resembles in nucleotide sequence at least a portion of class 1 RNA tumor virus genomes (*39*).

A model for the creation of an RNA tumor virus genome by paraprocessing is presented in Fig. 3 and is compared to the usual RNA processing from a comparable class 1 gene in a differentiated cell. The class 1 gene can be divided into two components, one that is repeated several times in the cell genome (R_1, R_2, ... R_n; where R_1 may or may not be identical to R_2) and one that is infrequently represented (I_1, I_2, ... I_n; where $I_1 \neq I_2 \neq I_n$) (Fig. 2). The initial virogene transcript is polycistronic and contains RNA sequences that are equivalent to both R and I virogene elements. During the usual type of RNA processing, many or all R sequences are cleaved from the RNA (Fig. 3). At some point the transcripts acquire a terminal poly(A) sequence—that is, before or after cleavage. Some of the RNA molecules may be destroyed completely. The surviving RNA is transported from the nucleus. After transport, the poly(A) segment is shortened by some 20 to 50 nucleotides then becomes progressively shorter with time, as suggested by Sheiness and Darnell (*33*).

If the cell genome or particular regulatory elements become altered in a particular fashion ("H" change), this series of events is modified so that paraprocessed RNA accumulates. The alteration produced by an "H" change prevents an early cleavage step in trimming the RNA transcript and thereby precludes some subsequent processing events. The alteration

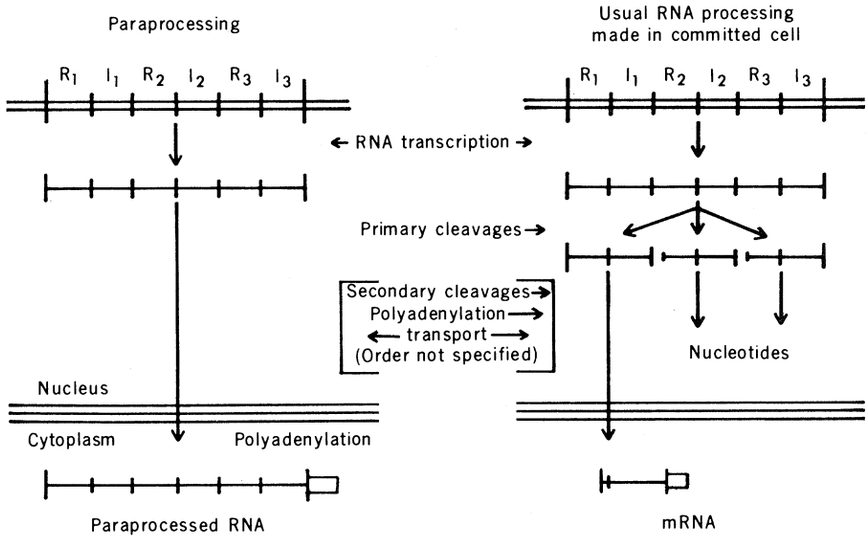


Fig. 3. RNA processing showing normal events and events leading to virogenesis: alternative methods for processing RNA transcripts from a class 1 gene. The modes of gene expression shown are consistent with but do not depend on regulation at the level of RNA synthesis. They are also compatible with the notion that large blocks of mammalian cell genome are transcribed at a particular time and that many of the transcripts are destroyed completely, some partially, and some almost not at all. RNA is indicated by a single horizontal line, DNA by two parallel lines, and the nuclear-cytoplasmic border by three parallel lines. The class 1 gene contains both R and I elements as described in the legend of Fig. 2, except that the R elements of the class 1 gene differ from one another. In the usual processing mode in committed cells a large RNA transcript is synthesized from a class 1 gene or from another genetic element. After synthesis, primary endonucleolytic cleavages occur. Much of the 5′ end of the cleaved molecules (R element) is destroyed, and the 3′ end consists of polyadenylic acid. The order of these events is not specified, but they occur in the cell nucleus. Some primary cleavage products are destroyed completely. The surviving polyadenylated RNA molecules are transported from the nucleus to the cytoplasm, the poly(A) is shortened, and the RNA is available or can be made available for protein synthesis. In paraprocessing, a large RNA transcript is synthesized from a class 1 gene. The transcript may undergo some nucleolytic attack, but the surviving RNA molecule is still large and contains both R and I elements. This incompletely cleaved RNA is then transported from the nucleus and, in the example shown, is polyadenylated in the cytoplasm. Among RNA transcripts synthesized at a particular time from a particular class 1 gene, some may be processed by the usual mechanism used by committed cells, others by paraprocessing.

does not prevent transport of the RNA from the nucleus to the cytoplasm. In the scheme of Fig. 3, the RNA acquires poly(A) in the cytoplasm and the poly(A) tract is not shortened as it is in the case of cellular mRNA.

Though the paraprocessing model for the genesis of RNA tumor viruses derives from knowledge of the physical structure of RNA tumor virus RNA, some aspects are supported by other data as well. In mouse hepatomas, RNA sequences complementary to repeated DNA sequences and normally restricted to the nucleus are released to the cytoplasm in an uncontrolled manner (*40*). This phenomenon can be duplicated with isolated nuclei and an adenosine triphosphate (ATP)–requiring RNA transport system. It also appears that RNA transport from isolated nuclei in a system requiring cytoplasm factors differs in nuclei derived from normal and tumor cells (*41*). Finally, the content of virus-specific RNA in the nucleus of mouse cells transformed by murine sarcoma virus is extraordinarily high (1 to 5 percent) (*42*). All these observations indicate a difference in RNA processing or transport (or both) between tumor cells and comparable normal cells and are consistent with a difference in an early cleavage step of RNA processing.

One result of the "H" change is the cytoplasmic accumulation of high-molecular-weight polycistronic RNA. The "H" change itself could take place in a "hot spot" element (*43*). The "hot spot" element is a readily mutable or frequently recombining genomic DNA site that, when altered, can affect the regulation of expression of viral genes or the action of viral gene products. The "hot spot" might be a site that controls RNA processing, although the original "hot spot" model does not restrict itself to gene control at this level. The "H" change in the RNA model can be a genetic alteration that directly changes the processing signals in the class 1 gene transcript or one that modifies the processing machinery. Alternatively, it might be an environmental or physiological (that is, hormonal) change that indirectly affects class 1 gene expression. Any metabolic disturbance that leads to paraprocessing of class 1 gene transcripts generates the potential of creating an RNA tumor virus genome.

In the example diagrammed in Fig. 3, the first accumulation of paraprocessed RNA is nuclear. The incompletely cleaved RNA is transported from the nucleus and then acquires poly(A) sequences. In the case of sea urchin embryos at the two- to four-cell stage 25*S* to 50*S* RNA containing long poly(A) stretches has been detected in the cytoplasm (*44*). Here the evidence indicated a cytoplasmic polyadenylation of high-molecular-weight maternal mRNA. Considering the proposed relation between embryogenesis and virogenesis (*24, 45*), we suggest that the creation of an RNA tumor virus genome may involve transport of particular RNA types from the nucleus of committed cells prior to their cleavage and full polyadenylation.

In the formation of paraprocessed RNA, it is not necessary that the processing alteration be an all-or-none event. The "H" change of Fig. 3 can reduce the probability that a given RNA transcript will be processed by the usual mechanism, rather than alter the processing of every class 1 gene transcript in that cell. Subsequent "H" changes can reduce the probability even further, as is discussed below. For example, if signals for RNA processing are particular nucleotide sequences in the RNA to be processed and if these sequences evolve so that RNA of different animals contain different signals, then an RNA tumor virus genome (a replicating paraprocessed RNA) introduced into a heterologous cell can recombine with host DNA and alter its class 1 genes. This may modify the RNA processing signals of those genes. This in turn may result in the establishment of a recombinant class 1 gene having a very low probability of usual expression, and hence a very high probability of paraprocessed RNA accumulation. This mechanism might explain why "xenotropic" viruses replicate poorly in cells of the species that generated them, but often grow well in cells from some other animals.

A recombination event between the genome of an infecting class 1 or 2 virus and that of the recipient cell provides one mechanism for the apparent rapid genetic change in RNA tumor viruses relative to change in classical cell genes (*46*). Preliminary evidence indicates that the infecting virus (class 1 or class 2) recombines specifically with genes in the recipient cell that are identical or related to class 1 viral genes (*47*). The genetic information acquired by simian or murine class 2 viruses from rat or primate cells or animals appears to be recombinant over short stretches (*48*), an argument against the simple "turning on" of endogenous (class 1) virus genes by the infecting virus. In the context of the RNA processing theory, this genotypic mixing can be obtained if, among the recombination events that took place during virus infection, there were some events affecting the processing of transcripts of a class 1 gene. It should be noted that, while the phenomenon of rapid genetic change of RNA tumor viruses is being increasingly observed (*46, 49*), there is evidence indicating that proteins coded by the virus genome do not evolve unusually rapidly (*22*). This dichotomy implies that the amino acid sequence of RNA tumor virus proteins is actively conserved, as is the amino acid sequence of most cell proteins, but that nevertheless the nucleotide sequence of the RNA tumor virus genome undergoes considerable variability. Unusually rapid nucleotide sequence variation in RNA tumor virus genomes during their formation from presumed cell progenitor genes is implicit in the protovirus (*45*), and hot spot (*43*) theories, and in these hypotheses the variation is linked to the evolution of a tumorigenic virus from precursor nontumorigenic agents.

As in other models (*24, 43, 45, 50*), the fate of a given paraprocessed RNA cannot be specified. It may remain intracellular and carry out no activities, other than a passive one, as a template of protein synthesis. The 35*S* (paraprocessed) form of tumor virus RNA does become associated with polyribosome-like structures in cells (*51*). Alternatively, it may become an intracellular self-replicating unit if the cell also contains reverse transcriptase. By all accounts, intracellular reverse transcriptase is a particulate cytoplasmic enzyme (*52*), and it appears that RNA of class 2 viruses codes for this protein (*53*). When paraprocessed RNA has this coding capacity, one of the proteins produced on a paraprocessed mRNA template could be the reverse transcriptase. Indeed, this mechanism is implicit in Temin's protovirus model (*45*). However, even if the paraprocessed RNA does not code for a replicating enzyme it need not be innocuous. Its fate and its effect on the cell may depend as well on other proteins coded by the class 1 genes, on the effect of cytoplasmic paraprocessed RNA on metabolic activities of differentiated cells, or on the regulation of translation of the paraprocessed RNA. When the coded proteins include those necessary for particle assembly but do not include reverse transcriptase, the cell may produce biologically inactive virus particles (for example, as from a so-called S^+ L cell) (*54*). When the proteins also include reverse transcriptase, the progenitor cell can produce replicating, infectious particles. When the proteins include those that adversely affect cell maturation, cell transformation may result.

The ability of a normal cell to produce replicating, infectious class 1 (or with sufficient genetic modification, class 2) viruses raises the possibility of transferral of virus genetic information from the normal gene pool of one animal species to that of another. This genetic transfer would be evidenced by a close genetic relation between the virus genome and genes in two dis-

tantly related animal species. The genome of the endogenous baboon virus and genes in normal baboons and cats are closely related (*55*), and also the viruslike nucleotide sequences in normal human white blood cells are related to those of Rauscher mouse leukemia virus (*47*). It will be important to establish whether the interspecies transfer of class 1 viral genes is unusually frequent and, if so, whether the transferred genes are utilized during embryogenesis to the advantage of the recipient animal. In this context, the interspecies transfer of class 2 viral genes would probably be deleterious to the recipient animal, leading, for example, to tumor formation (*56*).

Class 1 Genes of Normal Cells

Class 1 genes are defined as those nucleotide sequences in the DNA of normal cells that hybridize to RNA of class 1 viruses. Class 1 genes need not be identical in nucleotide sequence to RNA isolated from an established virus since the virus may undergo change. There may exist genes in normal cells which correspond to a portion of class 2 virus RNA, but this is difficult to assess, presumably because of virus-cell divergence.

Two structural properties of class 1 genes are shown by the information presented above. (i) The class 1 genes correspond to a large part of the information of established class 1 virus genomes. (ii) Class 1 genes are divided almost equally into R and I DNA sequences, whereas most genes that code for mRNA are I DNA sequences. (iii) The hybridization of RNA from established class 1 viruses to R sequences in DNA from normal cells is species-specific (Table 3) (*10*). In the most striking case, RNA from MMTV hybridized to R sequences in DNA from mice but not to sequences from rats and other mammals (*10*). In other cases, RNA from RD114 annealed only to R sequences in DNA from cats and not to those from other mammals; and RNA from RAV_0 hybridized to R sequences in DNA from chickens but not to those from quails (*10, 11*). Hybrids formed at low temperature between class 1 virus RNA and R sequences in DNA from normal progenitor cells have the same high thermal stability as hybrids formed at considerably higher temperatures (Fig. 4). This behavior is not typical of hybrids formed between mammalian cell RNA and R sequences in homologous cell DNA, for with cell RNA the thermal stability of the RNA-DNA hybrid is directly related to the temperature of hybrid formation (*8*). RNA from these class 1 viruses, therefore, anneals to special R sequences in progenitor cell DNA, special in that they are sequences restricted to a species or to a small group of animals and special in that they are an unusually distinct set of sequences within the progenitor cell genome. DNA sequences with these same properties have been detected in the mouse genome (*57*).

The hybridization results show that the R element of class 1 viral genes in uninfected progenitor cells are an informationally distinct set. The species-specificity of these viral sequences may be intimately related to the biological differences between animals; they may be involved in steps of developmental processes. The notion that class 1 genes are involved in normal differentiation and virogenesis is consistent with the proposed relation between modification of these genes and leukemogenesis as a block in differentiation (*43, 45, 58*).

There must be in normal cells several different sets of genes capable of generating RNA tumor viruses. In mice, three or four types of virus information can be discerned by molecular hybridization: MMTV's, two classes of leukemia viruses (the Friend-Rauscher-Moloney group as compared to the Gross type), and at least one type of sarcoma virus information (*6, 7, 10, 54*) (Table 2). Similar experiments with nucleic acids of virus particles from cats or primates show at least two types of virus information; "endogenous" viruses bear little or no homology to the known sarcoma or leukemia viruses (*13, 59*). Also, proteins such as reverse transcriptase and those that bear group-specific (gs) antigens from endogenous cat or primate viruses have little or no demonstrable immunologic relation to similar components of sarcoma or leukemia viruses from the same animals (*13, 22*). These informational differences contrast with the structural similarities among the viruses. These similarities are: (i) particle morphology; (ii) presence of a reverse transcriptase; (iii) physical structure of the genomic RNA; and (iv) number and size of the major viral proteins (*7*).

Table 3. Hybridization of RNA from class 1 viruses to repeated sequences in DNA from different animals. Hybridization was carried out between 0.5 ng of viral RNA and 50 μg of cell DNA. Reactions were terminated at a C_0t of 10^3. See legends to Fig. 1 and Table 1 for experimental details.

Source of RNA	Source of DNA	Percent RNA hybridized at $C_0t = 10^3$
Mouse mammary tumor virus, strain DW	Mouse	45
	Rat	0
	Cat	0
	Rabbit	1
	Calf	0
	Human	0
Cat virus, strain RD114	Cat	40
	Rat	0
	Mouse	3
	Calf	2
	Human	0
Mouse ribosomal RNA	Mouse	76
	Rat	74
	Calf	77
	Human	73
Mouse poly(A)-containing RNA (cytoplasmic)	Mouse	45*
	Rat	37*
	Human	15*

* Carried out to a C_0t of 10^4.

It can be argued that the informational dissimilarities described above arise from a common ancestor through divergent evolutionary pathways; the frankly tumorigenic sarcoma and leukemia viruses arising from the more host-related "endogenous" viruses. Were this true, the similarities among leukemia viruses from different species should not be as great as the similarity between "endogenous" and leukemia viruses from the same species of animal. Studies on the nucleotide sequence of the RNA genomes of relevant viruses by molecular hybridization and work on the relatedness of viral proteins by immunological methods show that among the avian and possibly murine viruses, the endogenous viruses examined so far are closely related to sarcoma or leukemia viruses (*54*), but this is not the case with feline and primate viruses (*22, 59*). The leukemia viruses from different animal species are, in fact, demonstrably related to one another (*6, 7*), while the endogenous viruses of felines and primates are not detectably related to leukemia or sarcoma viruses from the same species (*22, 59*).

These diverse findings can be reconciled by postulating that several different class 1 genes exist in a normal cell. Some class 1 genes give rise through "misevolution" (*45*) to the genomes of sarcoma-leukemia viruses; some cannot, but can give rise to other types of viruses, for example, carcinoma viruses. Viruses can be transferred from one species to another as class 1 viruses (*55*), then they may become class 2. We believe that simian sarcoma virus originated in this way.

We propose that class 1 genes are intimately involved in the normal differentiation process and that creation of genuine tumor viruses from them results from their misuse. They are expressed transiently during development when the activity of their gene products is essential for a particular biochemical activity. In this situation their expression is effected by paraprocessing of the RNA transcript. If these

genes need to be expressed in a differentiated cell, or a cell committed to a certain differentiation pathway, their RNA transcripts are ordinarily processed by the mechanism which that cell used to process the remainder of its RNA. Occasionally, the transcripts are accidentally paraprocessed in the committed or differentiated cell, and this accident creates the potential for the formation of an RNA tumor virus genome.

Virus Markers in Human Cancer

Proteins and nucleic acids genetically related to analogs obtained from purified woolly monkey sarcoma-leukemia virus or gibbon ape leukemia virus (the simian RNA tumor virus family) have been detected in cells of patients with acute myelogenous leukemia (AML) [see (*7*) and (*47*) for more complete reviews]. These molecular species may also be present in some persons that never develop cancer, albeit less frequently than in persons with leukemia. Cells from AML patients contain paraprocessed-like RNA [high molecular weight; contains poly(A)] (*60*), some of which can serve as a template for the endogenous synthesis of DNA sequences by cytoplasmic viruslike particles (*61–63*). These DNA sequences are related to RNA from the woolly monkey sarcoma-leukemia virus grown in rat cells or human cells (*47, 61*) and to RNA from Rauscher leukemia virus (*47, 61–63*). The reverse transcriptase capable of carrying out this reaction has been purified from cells of AML patients and shown to be immunologically related to the reverse transcriptases of the simian RNA tumor virus family (*52*).

White blood cells from normal humans also contain cytoplasmic particles that carry out endogenous synthesis of DNA with viral sequences, but this reaction occurs primarily on a DNA-template, and the newly synthesized DNA sequences are related to RNA from woolly monkey virus grown in human cells and to RNA from Rauscher leukemia virus, but not to RNA from woolly monkey virus grown in rat cells (*47*). Examination of the DNA sequences synthesized by cytoplasmic particles of normal cells in the presence of high doses of actinomycin D has provided evidence for DNA synthesis on an RNA template (*64*), and also in one for ordering DNA sequences complementary to RNA from woolly monkey virus grown in rat cells (*65*). Finally, a protein related to the p30 protein of the simian RNA tumor virus family has been detected in some patients with acute leukemia (*66*), but it has been reported that a similar protein is also detectable in cells from normal persons (*67*). The implications of these findings must wait until the human p30 proteins are purified and the specificity of the viral probes is completely evaluated. Nevertheless, these results suggest that a class 2 RNA tumor virus is present in cells of at least some patients with AML, and they indicate further that the viral components may not necessarily be confined to neoplastic cells.

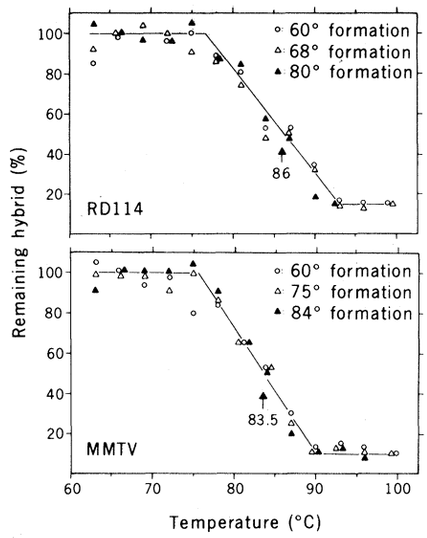


Fig. 4. Thermal stability as a function of temperature of formation of hybrids between RNA from class 1 viruses and repeated sequences in DNA from uninfected natural host cells. Hybrids were formed as described in the legend to Fig. 1, except that only 50 μg of DNA was used per 0.5 ng of RNA, and the reactions were terminated at a C_0t of 10^3. The detection of hybrids in hybridization mixtures is also the same as described for Fig. 2, except that the hybridization mixtures were exposed (in 0.15*M* NaCl plus 0.015*M* sodium citrate) to the indicated temperatures for 5 minutes prior to ribonuclease treatment. The results are given as percentages of a control incubated for 5 minutes at 0°C (45 percent of the input RNA). MMTV, mouse mammary tumor virus; RD114, a cat RNA tumor virus.

The ability of human tumor cells to release virus particles although of interest is usually a rare and transient occurrence (*68*). However, in one case myeloid cells from a patient with AML were cultured for several weeks under conditions that allowed the cells to maintain their differentiated state and to grow exponentially. These cells released C type RNA tumor virus particles containing reverse transcriptase immunologically related to analogs isolated from the simian RNA tumor virus family (*69*). These virus particles have high-molecular-weight poly(A)-containing RNA, related in nucleotide sequence to RNA from the simian RNA tumor virus family (*70*). Although this virus was obtained repeatedly from cells of one patient, cells from 17 other patients were cultured and analyzed similarly but they failed to yield detectable virus even when components related to the simian virus family were sometimes detected in the fresh cells (*71*). In the RNA paraprocessing framework, tumors can develop with or without complete virus production or even without many of the markers described earlier; however, they should all be associated with paraprocessing of the virus RNA.

What then is the genetic origin of the virus and viruslike components found in human AML cells? Although some points of the evidence appear self-contradictory, there is agreement on one important question; if a human virus involved in AML is closely related genetically to the simian RNA tumor virus family, then the human virus (in its entirety) cannot be endogenous to humans in the true sense of the word "endogenous." In this context an endogenous RNA tumor virus should carry nucleotide sequences also found in the germ line of all individuals from a given species. In the simplest case, the genes would be found in all tissues of adults of that species, and it is in this case that nucleotide sequences of viruses of the simian family cannot be endogenous to man. Neither the RNA isolated from members of the simian virus family (*7*) nor DNA copies of this RNA (*72*) significantly hybridize to DNA from placenta or white blood cells of normal humans under stringent conditions of hybrid formation. Furthermore, no simian virus–related genes have been found in circulating blast cells from patients with AML (*7, 72*). If the provirus is present in these cells, either it is not frequent enough to be detected (less than one copy per ten cells) or it is related to only a small portion of the RNA (less than 10 percent). Genes in leukemic cells that hybridize to the DNA sequences synthesized endogenously by cytoplasmic particles from the same cells have been detected (*73*) and may be cytoplasmic genes (*7*), but they have not been identified as coding for the human proteins related to simian virus. We conclude from this evidence that RNA tumor viruses related to simian virus are not completely endogenous to human viruses nor is a provirus related to simian virus present in most of the morphologically transformed cells of persons with AML. This would mean either that a provirus in the transformed blood cells is not sufficiently related to nucleotide sequences of simian viruses for detection or that a provirus related to simian virus exists in some cells, and products of the expression of this provirus secondarily transform other blood cells.

Summary

The results of molecular hybridization experiments with high-molecular-weight RNA isolated from RNA tumor viruses and DNA from normal cells suggest that RNA tumor virus genomes originate from cell genes. Some RNA tumor viruses (here called class 1) appear to have been generated in recent times in that their RNA is closely related in nucleotide sequence to certain cell genes (class 1 genes). A second class of RNA tumor viruses (here called class 2) is more distantly related to genomic information of normal cells. Structural properties of the RNA of RNA tumor viruses lead us to propose that the tumor virus RNA is originated when RNA transcripts of class 1 genes are processed by a mechanism we call "paraprocessing." We postulate that RNA paraprocessing is normally used only at particular times during differentiation and is characterized by the cytoplasmic appearance of high-molecular-weight RNA chains containing terminal polyadenylic acid (200 residues). Paraprocessing of class 1 gene transcripts in committed or differentiated cells is considered to be aberrant in transcription that can lead to the generation of an RNA tumor virus genome. If the paraprocessed class 1 gene transcript codes for a reverse transcriptase, replication of the RNA becomes possible. Transfer of the replicating RNA to a new cell can result in genetic change such that the virus genome mutates, differing from the original progenitor genes. We propose that this genetic change causes class 1 viruses to become class 2. These ideas are applied to evidence concerning the biology of infection of RNA tumor viruses and concerning the involvement of RNA tumor viruses in human cancer.

Genetic change can also occur during the origination of an RNA tumor virus genome by repeated reverse transcription and recombination (*45*) or by genetic alteration of particularly changeable cell genes ("hot spots") (*43*).

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NEWS AND COMMENT

Fetal Research: Ethics Commission Votes to End the Moratorium

The "four month" moratorium on research on the living human fetus, which legally has been in effect since 12 July of last year,* should be lifted to allow such research to go on under carefully circumscribed circumstances. This, in essence, is the recommendation that the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research has sent to the Secretary of the Department of Health, Education, and Welfare (HEW).

After studying and debating the ethical, legal, and scientific issues relevant to research involving live fetuses, the commission has adopted a position that can be described as being moderately liberal. For example, the commission voted unanimously to allow experimentation on fetuses in anticipation of abortion and, with only one dissent, implicitly acknowledged that there are situations in which one would want to single out for studies fetuses that are scheduled to be aborted rather than jeopardize those that will go to term.

Although it is likely that the Secretary will adopt the commission's recommendations generally as written, it is less certain that biomedical scientists, intimidated as they were by the very fact that Congress demanded a moratorium on fetal research, will rush forward with proposals for studies involving live human fetuses.

The commission, created under a section of the National Research Act of July 1974, was given a mandate to investigate a number of areas of research involving human subjects, but was instructed by law to deal with fetal research first. A commission staff paper recognizes that the priority given fetal research in the law is indicative of "the concern of an overwhelming majority of the members of Congress that unconscionable acts involving the fetus might have been performed in the name of scientific inquiry."

By design the 11 members of the commission were chosen to represent a variety of points of view and religious persuasions, as indeed they do. Five months elapsed between the time the commission was created and its members were actually appointed, during which time every special interest group one can think of lobbied the Secretary to appoint its favored candidates. Then, when the commissioners were finally named in December (*Science*, 27 December 1974), charges were made that the body was top-heavy with Roman Catholics and that it was stacked with persons who were antiscience. It appears, however, that those allegations cannot be supported. In spite of the conservative nature of many commissioners, their recommendations seem calculated neither to please the Pope nor to call a halt to fetal experimentation. Generally, their recommendations are entirely reasonable.

The commission covered a lot of territory in its deliberations on research on live fetuses. It contracted with Maurice Mahoney of Yale University for studies of the extent, nature, and purposes of fetal research worldwide, during the past 10 years. His survey revealed 3000 papers on the subject and showed that less than 1 percent of the research involved living fetuses after delivery. It contracted for a study of available medical technology for preserving the life of fetuses born at an early gestational age, as part of its efforts to define what a "viable" fetus is. The study, headed by Richard Behrman of Columbia University, was summarized by the commission staff in a brief paper which concluded that "On an empirical basis the current limits of viability are clear: an infant born weighing less than 601 grams at a gestational age of 24 weeks or less has never survived."

The commission solicited papers from legal scholars, as well as from ethicists and philosophers. It held a day of hearings at which public witnesses testified. And, it consulted its own expertise—the commissioners themselves are medical researchers, lawyers, and ethicists—in arriving at its conclusions.

From the start, commission chairman J. Kenneth Ryan† of Harvard Medical School, was committed to the idea of achieving consensus on these recommendations about fetal research, which may be the most sensitive and difficult the group will have to make. Particularly because fetal research is so closely tied in many persons' minds to the abortion conflict, it seemed evident that the force of the recommendations would be seriously diluted were they to be accompanied by very many minority reports. Throughout the day-long session during which they voted on recommendations, Ryan urged commission members to first handle those portions of issues on which they could agree and then tackle areas of disagreement. As a result,

*For all practical purposes, there has been a ban on research on living fetuses since April 1973, when officials of the National Institutes of Health promised a contingent of Roman Catholic schoolgirls that they would not support such experimentation.

†Ryan was elected chairman by the commissioners themselves after their first meeting.