with lethal haplotypes are presumed to cease expression by 9 days of development. Furthermore, as *in vitro* populations of multipotential teratoma cells differentiate, detectable levels of F9 antigen fall, while H-2 shows a concomitant rise[29].

Sixth, both systems seem to have analogues in other species. MHC complexes like *H-2* have been found in all mammals studied, as well as birds[21]. Likewise, antigens cross reacting with F9 ($+t^{12}$) have been found on all mammalian sperm examined[30], including human sperm[31,32].

Finally, recent biochemical evidence suggests that the antigenic products of both *T/t* and *H-2* are structurally similar. Lactoperoxidase radioiodination of cell surface components of lymphocytes, sperm and F9 cells, and immunoprecipitation with appropriate antisera, show not only that the molecular weight and subunit structure of the D and K products of *H-2* and the F9 ($+t^{12}$) antigen are identical, but also that $+t^{12}$, like H-2, is associated with a B-2-microglobulin-like moiety in the membrane[25]. Similar results have been reported for the TL antigen[33,34] which is closely linked to *H-2D* and apparently has a reciprocal interaction with it in the plasma membrane. Amino acid sequencing of $+t^{12}$ is now under way and together with sequence data for H-2, will prove or disprove the homology strongly suggested by these data.

The considerations above suggest that the *T/t* complex may be an evolutionary precursor of the MHC, or that both complexes originated in a common ancestoral gene. An embryonic recognition mechanism of the type associated with *T/t* genes could have evolved with the metazoans at the stage when an immune system was not yet organised, indeed, not yet necessary. Later, with the advent of vertebrates and especially warm blooded animals, when protection from microorganisms became imperative, the duplication and specialisation of a complex genetic region already governing cell recognition may have been an economical way of providing genes for immunological recognition.

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articles

Genetic control of cell size at cell division in yeast

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A temperature-sensitive mutant strain of the fission yeast Schizosaccharomyces pombe *has been isolated which divides at half the size of the wild type. Study of this strain suggests that there is a cell size control over DNA synthesis and a second control acting on nuclear division.*

GROWING cells tend to maintain a constant size at division[1–3]. This implies that there is coordination between cellular growth and cell division which results in a division occurring when the cell has grown to a particular size. The mechanism by which this is achieved remains obscure although there are various theoretical models which could account for the phenomenon[4]. A new approach to the problem would be the study of mutant strains in which the normal coordination between cellular growth and cell division is disrupted, resulting in the division of cells at different sizes. Such an approach in a simple eukaryote is described in this report.

A mutant strain of the fission yeast *Schizosaccharomyces pombe* has been isolated which divides at half the wild-type size. Study of this strain demonstrates that its cell cycle is also profoundly altered compared with wild type. The timing of DNA synthesis in the cell cycle, the size at which cells undergo DNA synthesis, and the size at which cells undergo nuclear division are all different from wild type growing with the same generation time. These observations may be understood in terms of models of cell cycle control that propose a cell size control over DNA synthesis and a second control acting on nuclear division.

Cell division cycle

The cell division cycle of fission yeast has been extensively studied[5,6]. Cell division is marked by the formation of a prominent cell plate across the middle of the cell, and separation of the two cells occurs a little later. The cell remains constant in diameter, growing only in length during the cell cycle. Cell length is therefore directly related to cell volume. As in higher eukaryotes the DNA–nuclear cycle can be divided into G1, S, G2 and M periods. The S period is very short and occurs at the beginning of the cell cycle

Table 1 Characteristics of wild type and mutant at 25 °C and 35 °C

Strain	Growth temperature (°C)	Generation time (min)	Cell volume at cell division (μm³) Mean	s.d.	Cell volume at nuclear division (μm³) Mean	s.d.	Macromolecular content per cell Protein (pg per cell)	RNA (pg per cell)
Wild-type 972	25	228	129	9.1	121	8.4	12.4	2.54
	35	142	149	6.4	139	9.0	14.1	2.90
Mutant *cdc*9–50	25	232	109	8.8	98.0	7.9	9.66	2.26
	35	138	72.7	7.8	60.4	8.4	7.16	1.54

Cultures of the two strains were grown in a modified EMM 2 minimal medium[5], with the sodium acetate and sodium dihydrogen orthophosphate replaced by 15 mM potassium hydrogen phthallate and 10 mM disodium hydrogen orthophosphate. Photographs of cells grown at 25 °C and 5 h after shift to 35 °C were taken using a ×16 objective in a Zeiss photomicroscope under dark field optics. The length and diameter of 200 cells with cell plates were measured and their volume calculated. Protein and RNA content per cell, and generation times were determined in exponentially growing cultures as described in ref. 12. Between 5 and 10 determinations were made for each estimate of macromolecular content per cell and in no case did the standard error exceed 4% of the mean. Cells with dividing nuclei were detected by staining with Giemsa[5]. The volume of 200 such cells was calculated after correction for cell shrinkage on fixation and staining.

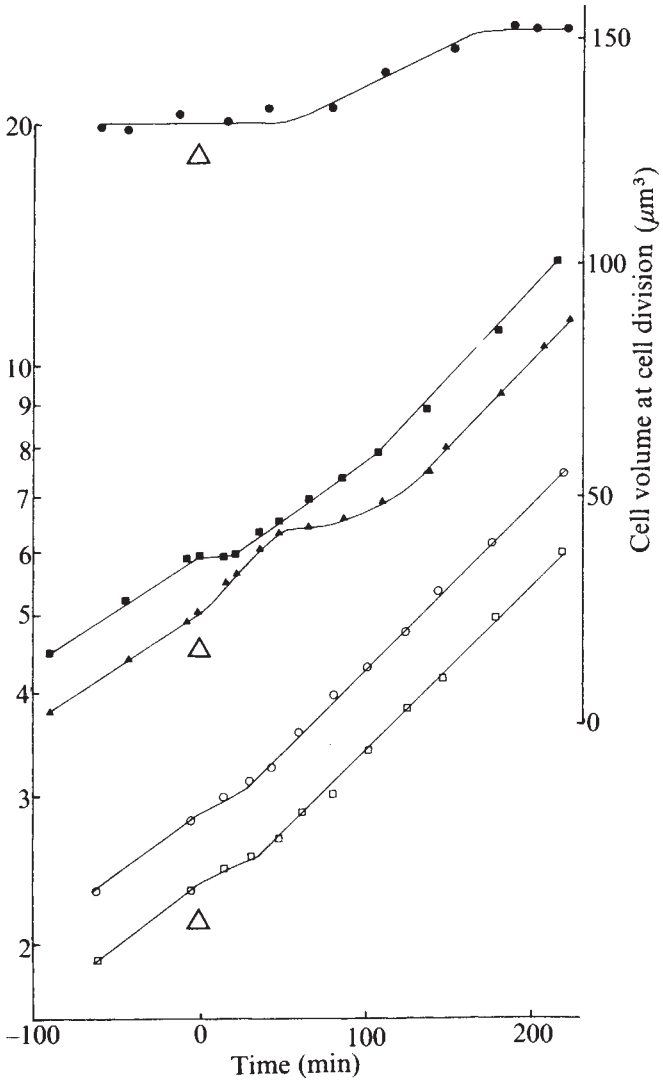

Fig. 1 Cell number, nuclei number, RNA, protein, and cell volume at division of wild-type 972 during the transition from exponential growth at 25 °C to 35 °C. A culture of 972 growing at 25 °C was shifted at 0 min to 35 °C, the culture reaching the higher temperature within 5 min of transfer. The time of transfer is marked by the large open triangles. Experimental parameters were determined as described in Table 1. The real values of experimental parameters per ml, equivalent to one unit on the arbitrary log scale, is given in brackets in the symbol key. ■, No. of nuclei ml^{-1} (1×10^6); ▲, cell number ml^{-1} (1×10^6) ;○, protein ml^{-1} (21.9 μg); □, RNA ml^{-1} (5.46 μg); ●, cell volume at cell division.

simultaneously with cell separation. A long G2 period follows culminating in nuclear division about three-quarters of the way through the cell cycle. There is then a short G1 period ending with the start of the S period of the next cell cycle.

Characterisation of mutant strain

The mutant strain *cdc*9–50 was isolated from wild-type *S. pombe* (972h$^-$ obtained from U. Leupold, Bern) after nitrosoguanidine mutagenesis (P. Nurse and B. Carter, unpublished). When grown at 35 °C, cells of strain *cdc*9–50 divide at about half the volume of wild-type cells at that temperature (Table 1). The mutant phenotype is temperature-enhanced since at 25 °C *cdc*9–50 divides at a cell volume only 15% smaller than wild type (Table 1). These differences are also reflected in the RNA and protein content per cell in exponentially growing cultures of the two strains at the two temperatures (Table 1). Thus *cdc*9–50 divides at both a lower cell colume and a reduced macromolecular content compared with wild type. In spite of these differences *cdc*9–50 has a similar generation time to wild type at both 25 °C and 35 °C (Table 1).

The mutant phenotype of reduced cell size at division segregated 2 : 2 in all of 27 asci dissected from a wild-type 975 h$^+$ × *cdc*9–50*h*$^-$ cross (P. Thuriaux, personal communication), demonstrating that it is caused by a mutation in a single nuclear gene.

Size control over nuclear division

Use was made of the temperature sensitivity of the mutant phenotype to determine the stage in the cell division cycle at which the coupling of cellular growth to cell division takes place. Exponentially growing cultures of *cdc*9–50 and wild type at 25 °C were shifted to 35 °C. Cell number in the wild-type culture continued to increase for 50 min, stopped for about 30 min, and then gradually increased to the normal growth rate at 35 °C (Fig. 1). During the short plateau in cell numbers, RNA and protein continued to increase, consistent with the increase in cell volume at division that is observed at this time (Fig. 1). The temperature shift temporarily inhibits nuclear division, since for 25 min after the shift there is no further increase in the number of nuclei per ml of culture, and the normal rate of increase at 35 °C is only reached after a further 70 min (Fig. 1). Those cells in which nuclear division is temporarily inhibited are delayed from entering cell division, accounting for the observed short plateau and reduced rate of increase in cell number.

On shifting the culture of *cdc*9–50 from 25 °C to 35 °C, cell number continued to increase for 50 min and then almost stopped, similar to the behaviour observed in wild

type (Fig. 2). Unlike wild type, however, the plateau in cell number was very short (10–15 min) and was followed by a rapid rise rather faster than the usual rate of increase at 35 °C (Fig. 2). This rapid rise in cell number was preceded by a dramatic drop in cell volume at division (Fig. 2). RNA and protein continued to rise as in wild type (Fig. 2) and thus the RNA and protein content per cell dropped to the reduced level observed in exponential culture at 35 °C (Table 1). For 20 min after the temperature shift there was no increase in the number of nuclei per ml of culture showing an inhibition of nuclear division similar to wild type (Fig. 2). Thereafter there was a very rapid rise for a period of 40–50 min (Fig. 2), much faster than the normal rate of increase at 35 °C. This suggests that nuclear division is initiated in a large proportion of the population after a period of about 20 min at the higher temperature.

These data can be explained in terms of a size control over the initiation of nuclear division. In wild type at 25 °C the cell grows to a critical size and nuclear division is then initiated. On shift to 35 °C this control is modulated so that the cell grows to a larger size before nuclear division can take place. In *cdc*9–50 the size control is altered so that at 35 °C nuclear division occurs in cells of a smaller size than wild type. On shift from 25 °C to 35 °C nuclear division is briefly inhibited by the same modulation that is observed in wild type. The mutant phenotype, however, rapidly expresses itself by reducing the cell size at which nuclear division is initiated. This results in nuclear division occurring immediately in that proportion of the cell population that were too small for initiation at 25 °C, but which were above the smaller critical size required at 35 °C. Thus cells that were too small to divide at 25 °C would be accelerated through the cell division cycle and would divide at the smaller size characteristic of growth at 35 °C.

If *cdc*9–50 has an altered size control over the initiation of nuclear division, then nuclear division should occur in cells of a smaller size compared with wild type. When grown at 35 °C cells of *cdc*9–50 containing dividing nuclei were about half the volume of similar cells of wild type (Table 1). At 25 °C cells of *cdc*9–50 containing dividing nuclei were only about 20% smaller than wild type (Table 1). These relative differences are similar to the differences observed in cell volume at cell division for the two strains at the two temperatures. This would suggest that the size control over nuclear division determines cell size at cell division.

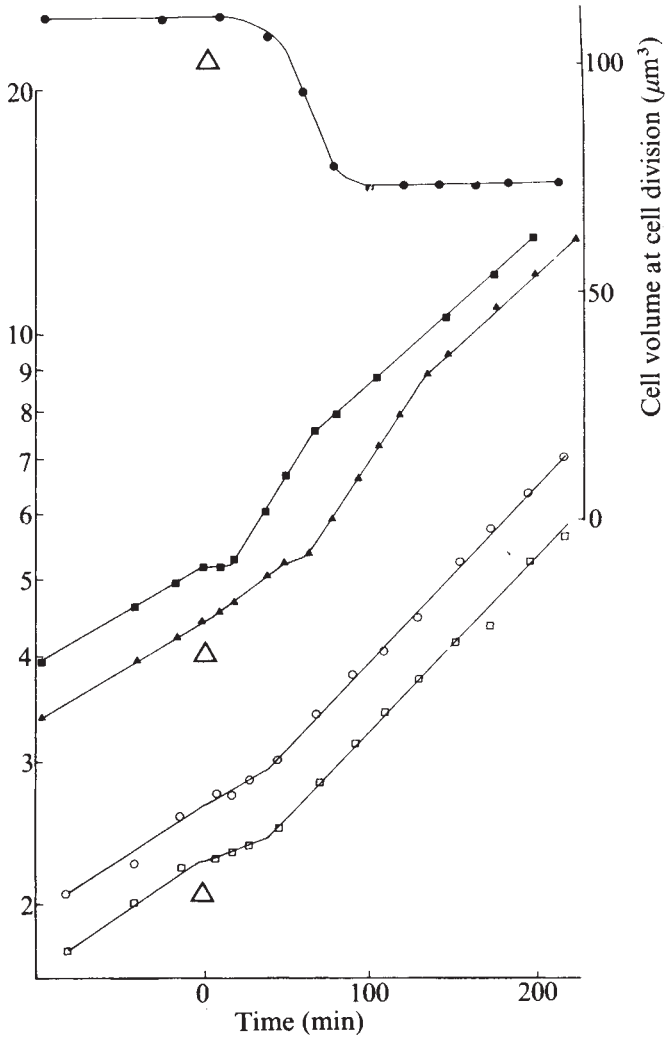

Fig. 2 Cell number, nuclei number, RNA, protein and cell volume at division of *cdc*9–50 during the transition from exponential growth at 25 °C to 35 °C. All experimental details as for Fig. 1. ■, No. of nuclei ml^{-1} (1×10^6); ▲, cell number ml^{-1} (1×10^6); ○, protein ml^{-1} (16.8 μg); □, RNA ml^{-1} (4.58 μg); ●, cell volume at cell division.

Timing of DNA synthesis during the cell cycle

The DNA content per cell in exponentially growing cultures is slightly reduced in *cdc*9–50 at 35 °C compared with wild type (Table 2). This suggests that a greater proportion of cells have the unreplicated 1C content of DNA in *cdc*9–50, and hence DNA synthesis occurs a little later in the cell cycle than in wild type. This was checked by measuring the increase in cell number in exponentially growing cultures after inhibiting DNA synthesis with hydroxyurea[2]. Under these conditions only those cells which have completed DNA synthesis can undergo cell division. The cell number increase for *cdc*9–50 was reduced compared with wild type (Table 2), again suggesting that DNA synthesis occurs later in the cell cycle in the mutant strain.

Table 2 Timing during the cell cycle and cell size at which DNA synthesis takes place in wild-type 972 and *cdc*9–50 growing at 35 °C

Strain	DNA content per cell in exponential culture (fg per cell)		Cell number increase after inhibition of DNA synthesis	Fraction of a cell division cycle at which midpoint of rise in DNA occurred	Macromolecular content per cell at midpoint of rise in DNA			
					Calculated		Measured	
	Mean	s.e.			Protein (pg per cell)	RNA (pg per cell)	Protein (pg per cell)	RNA (pg per cell)
Wild type 972	34.6	1.4	× 2.04	0.00	10.2	2.09	12.0	2.61
Mutant *cdc*9–50	28.4	0.77	× 1.86	0.29	6.29	1.35	5.87	1.37

Strains were grown at 35 °C as described in Table 1; DNA, protein and RNA content per cell were determined as described in ref. 12. Inhibition of DNA synthesis was achieved by the addition of 11 mM hydroxyurea. Three synchronous cultures were prepared as described in Fig. 3 and the mean fraction of the cell cycle at which the midpoint of rise in DNA occurred was determined (cell separation was taken as 0). The macromolecular content per cell at this time was measured, and was also calculated using the method described in ref. 8 and the data from Table 1.

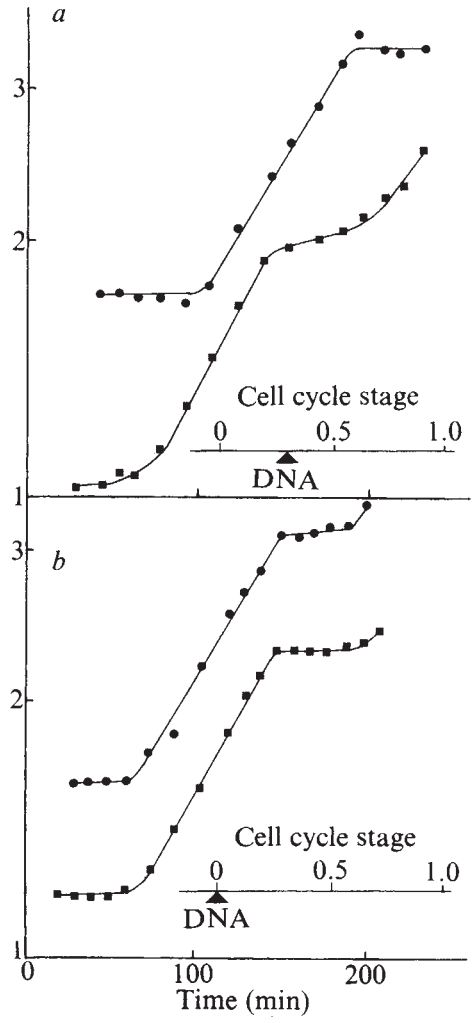

Fig. 3 DNA and cell number during synchronous cultures at 35 °C of *cdc*9–50 (*a*) and wild-type 972 (*b*). Strains were grown at 35 °C as described in Table 1. Small cells were selected from these cultures by sedimentation rate centrifugation through a lactose gradient in a zonal rotor[7]. The small cells were used as an innoculum for synchronous cultures and DNA and cell number determined, as described in ref. 12. A cell cycle map is given for both cultures starting at cell separation and showing the time of the midpoint of the rise in DNA. The real values of the experimental parameters per ml, equivalent to one unit on the arbitrary log scale, is given in brackets in the symbol key. *a*, ●, DNA ml^{-1} (8.63 ng); ■, cell number ml^{-1} (4×10^6). *b*, ●, DNA ml^{-1} (7.47 ng); ■, cell number ml^{-1} (3×10^6).

These observations were confirmed by synchronous cultures of *cdc*9–50 and wild type at 35 °C. Synchronous cultures were prepared by selection of small cells from an exponentially growing asynchronous population using sedimentation rate centrifugation through a lactose density gradient[5,7]. In wild type the midpoint of the rise in DNA occurred at the beginning of the cell cycle simultaneously with cell separation (Fig. 3, Table 2). This timing is similar to that found in wild type at 32 °C (ref. 6). In *cdc*9–50, however, the midpoint of the rise in DNA occurred at 0.3 of a cell cycle, about 40 min later than cell separation (Fig. 3, Table 2). Thus in *cdc*9–50 the G1 period is substantially lengthened at the expense of G2.

Knowledge of the timing of DNA synthesis during the cell cycle, and of the protein or RNA content per cell of an exponentially growing culture enables the protein or RNA content of cells undergoing DNA synthesis to be calculated[8]. Assuming protein and RNA to be accumulated exponentially during the cell cycle[9], the protein and RNA content of cells undergoing DNA synthesis has been calculated for 972 and *cdc*9–50 at 35 °C (Table 2). It can be seen that the macromolecular content of these cells is much reduced in *cdc*9–50 compared with wild type. This was confirmed by measuring the actual protein and RNA content of cells at the midpoint of the rise in DNA in the synchronous cultures (Table 2).

These results demonstrate that the mutant strain shows several differences to wild type. The timing of DNA synthesis in the cell cycle, the size at which cells undergo DNA synthesis, and the size at which cells undergo nuclear division are all different from wild type growing under identical conditions with the same generation time. Thus the single genetic lesion in *cdc*9–50 has profoundly altered the controls acting on a number of events occurring during the cell division cycle.

Model of cell cycle controls

To explain the pleiotropic behaviour of *cdc*9–50 and the maintenance of a constant cell size at division, it is necessary to invoke controls that act at both nuclear division and DNA synthesis. One such model would be that a cell size control acts over the initiation of nuclear division, and a second separate size control acts over an event required for initiation of DNA synthesis. The latter size control refers more precisely to a critical mass per genome which in wild-type cells is attained in the cell cycle previous to that in which DNA synthesis actually occurs. This is because the size control over nuclear division produces daughter cells that are already larger than the threshold size required to initiate DNA synthesis. Since nuclear division is required before DNA synthesis can take place (ref. 10 and my unpublished results using temperature-sensitive mutants defective in nuclear division), DNA synthesis cannot occur early in the previous cell cycle at the threshold cell size, but will be delayed until after nuclear division. Therefore the size control over DNA synthesis would not be expressed. In *cdc*9–50 the genetic lesion reduces the cell size at which nuclear division occurs, but does not alter the size control over the initiation event required for DNA synthesis. In this situation the size control over nuclear division produces daughter cells smaller than the threshold size required to initiate DNA synthesis. The cell then has to grow for a further 0.3 of a cell cycle before it is large enough for DNA synthesis to take place. In wild type the size control over DNA synthesis may be expressed only when the cell is growing in adverse conditions. For example, on approaching stationary phase, yeast cells tend to divide at a smaller size[5,11,12], and so the size control over DNA synthesis would be expressed. The operation of the size control would then prevent a new DNA synthesis–nuclear division cycle being started, accounting for the observed accumulation of stationary phase yeast cells in the G1 period of the cell cycle[11,12].

It should be noted that nuclear division could seem to be under a size control if there was a timing mechanism maintaining a constant period between the initiation event required for DNA synthesis and nuclear division. If the genetic lesion in *cdc*9–50 resulted in a shortened period compared with wild type, then nuclear division would be initiated earlier when the cell had grown less, and hence cell division would take place at a smaller size. In this situation which is directly analogous to that proposed for the control of the cell cycle in bacteria[13], *cdc*9–50 should be considered as altered in a timing mechanism rather than in a cell size sensing mechanism.

Thus fission yeast seems to maintain coordination between growth and cell division with cell size controls over DNA synthesis and nuclear division. The latter size control could, however, also be the consequence of a constant timing period between DNA synthesis and nuclear division.

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Infective transmission and characterisation of a C-type virus released by cultured human myeloid leukaemia cells

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A C-type virus isolated from long term cultures of myeloid cells from a patient with acute myelogenous leukaemia is infectious for a wide variety of cells. The establishment of chronically infected cells enabled us to characterise the virus by biological, immunological, and biochemical tests. The virus is closely related to the simian sarcoma-associated virus isolated from a woolly monkey fibrosarcoma.

THE possibility that C-type RNA tumour viruses are involved in human neoplastic disease has been the subject of much experimentation and speculation recently. In particular, human leukaemia has come under close scrutiny because so many leukaemias from diverse species of animals such as chickens, cats, and mice, have a viral aetiology[1,2]. Although viraemia does not accompany human leukaemia, it is possible that a C-type virus exists in a latent state and some of the well-known 'footprints' of C-type viruses have been detected in a variety of leukaemic tissues. These include: (1) cellular nucleic acid sequences homologous to viral RNA[3-7]; (2) presence of RNA-directed DNA polymerase (reverse transcriptase) activity akin to the viral enzyme[3,8-14]; (3) presence of intracellular proteins that cross react in serological tests with antibodies to virus-specific proteins[15,16]; and infrequently (4) electron microscopic identification of virus-like particles[10,12,17,18]. In many of these reports the virus-like components in the fresh human leukaemic cells were shown to be most closely related to similar components from oncogenic primate RNA tumour viruses, especially woolly monkey (simian) sarcoma virus (SSV)[4,5,7,14-16].

Gallagher and Gallo[19] have reported the production and preliminary characterisation of C-type virus particles from long term cultures of myeloid cells from one patient, HL23, with acute myelogenous leukaemia (AML). The uncultured blood cells of this patient contained reverse transcriptase related to that of SSV (ref. 14). The virus released by HL23 cells is called HL23V-1. In addition, they have now reported the release of similar particles from cells derived from a bone marrow specimen from the same patient obtained 14 months after the original blood specimen[20]. This virus is called HL23V-5.

Here we show that these C-type particles are infectious. The virus may be propagated to high titre in several cell types and a quantitative bioassay is described. The production of substantial titres of virus has enabled us to characterise the virus in detail and to compare it with and show that it closely resembles the C-type SSV and its associated virus (SSAV) previously isolated from a fibrosarcoma of a woolly monkey[21].

Infective nature of virus particles

Supernatant fluids from the AML peripheral blood cell cultures containing virus-like reverse transcriptase activity were initially inoculated on to a wide variety of animal and human cells. After transient bursts of viral replication over a period of 3 months, four chronically infected cell lines were established in A204 cells (human rhabdomyosarcoma), WHE2 cells (fibroblastic strain from a whole human embryo), A7573 (canine thymus) cells, and NRK (normal rat kidney) cells. The establishment of chronically infected virus-producing cells was therefore neither a frequent nor a rapid event. A more rapid transmission of the virus to two cell lines (A204; and KNRK, a rat kidney cell line transformed by, but not producing, Kirsten murine sarcoma virus) was obtained, however, within 3 weeks of exposure of these cells to β-propiolactone-inactivated Sendai virus immediately before inoculation with supernatant fluids from the AML cell cultures. Table 1 shows the variety of cells infected with the AML culture fluids and the frequency of infectious transmission to new host cells. The viruses produced by these new host cells are referred to as 'secondary' viruses; the nature of the host producing each secondary virus is indicated in parentheses.

The secondary HL23V-1 viruses will infect a wider variety of cells than the primary virus (Table 2). This may be a result of the virus acquiring a genuinely wider host range or more likely the result of selection by infective transmission of higher titre virus stocks and higher infectivity to particle ratios. Electron microscopy of chronically infected cultures revealed abundant C-type virus particles (Fig. 1). In contrast to the host range of SSAV, most of the HL23V-1 isolates infected 1283 marmoset cells poorly and human cells well (Tables 1–3).