ATLL in people represents a strong case for an etiological relation between a given virus and a human cancer (*5, 16*). The reported cases of lymphoid malignancies in macaques are not numerous (*17, 18*). However, there have been reports of multiple cases that occurred over brief intervals of time, suggesting that a transmissible agent could be involved (*14, 18, 19*).

The LPD observed in macaques have not been characterized according to the subpopulations of lymphocytes involved. Nevertheless, the seroepidemiological pattern we observed parallels that seen in people where the presence of antibodies to HTLV is associated with a greatly increased risk for the development of a particular form of lymphoma. Our current results indicate that an agent similar to HTLV is present in colonies of macaques where outbreaks of ML have been reported, and that more consideration should be given to the possible involvement of these agents in the causation of lymphoproliferative abnormalities in macaques.

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Nuclear Localization and DNA Binding Properties of a Protein Expressed by Human c-*myc* Oncogene

Abstract. *Antisera to the human cellular* myc *oncogene product were used to identify a human c-myc specific protein with a molecular weight of 65,000. Subcellular fractionation showed that the human c-myc protein is predominantly found in the cell nucleus. The p65K$^{c\text{-}myc}$ protein binds to double- and single-stranded DNA as measured by a DNA affinity chromatography assay.*

The oncogene v-*myc* of the avian myelocytomatosis viruses arose from a closely related gene (c-*myc*) found in all vertebrate animals (*1*). Altered expression of the c-*myc* gene is an important event in malignant cell transformation. Possible mechanisms whereby changes in c-*myc* gene expression have occurred include insertion of a strong retroviral promoter in the vicinity of c-*myc* (*2*), amplification of c-*myc* DNA (*3*), or chromosomal translocations which bring the c-*myc* gene in close vicinity to various immunoglobulin loci (*4*). The protein product of the v-*myc* oncogene has been studied in MC29 viral infected or transformed cell lines using an antiserum to the NH_2-terminal part of a polyprotein containing a v-myc protein at its COOH-terminal end. The MC29 transforming specific protein, p110K$^{gag\text{-}myc}$, with a molecular weight of 110,000 (110K) is a nuclear, phosphorylated protein that binds to double-stranded DNA (*5*). Subsequent studies with antisera specific for the protein product of the v-*myc* oncogene itself confirmed the results on the p110K$^{gag\text{-}myc}$ polyprotein and extended them to show nuclear localization for v-myc proteins unlinked to any structural viral antigen (*6*).

We used antisera to the human c-myc protein to identify a 65K protein expressed by the human c-*myc* gene (*7*). The p65K$^{c\text{-}myc}$ protein is expressed early in development and is evolutionarily conserved between different species. Increased levels of the p65K$^{c\text{-}myc}$ protein, as compared with normal cells, were observed in cells with amplified c-*myc* DNA or cells containing chromosomal translocations involving the c-*myc* gene (*8*). These results imply that the p65K$^{c\text{-}myc}$ protein plays an important role during c-*myc* oncogenesis. Furthermore, we showed that the p65K$^{c\text{-}myc}$ protein is an inducible gene product in the sense that synthesis of this protein is greatly enhanced after activation of cells with mitogens. We now report that the human p65K$^{c\text{-}myc}$ protein has two properties in common with the MC29 viral transforming polyprotein, believed to be relevant for viral transformation.

Three different antisera to human c-myc protein were used for the identification of the p65K$^{c\text{-}myc}$ protein (*7*). One of these anti(c-myc N-ter) was a rabbit antiserum to a synthetic peptide representing amino acids 24 to 40 of the human c-myc protein. The other two were raised against c-*myc* antigens expressed as fusion proteins in *Escherichia coli* containing either amino acids 42 to 180 anti(c-myc 5′ exon) or amino acids 380 to 439 anti(c-myc C-ter) of the human c-myc protein. Two of these antisera readily immunoprecipitated the p65K$^{c\text{-}myc}$ protein whereas the other, anti(c-myc 5′ exon), only weakly immunoprecipitated this protein. In addition, anti(c-myc 5′ exon) immunoprecipitated a 49K pro-

tein. The 49K protein was apparently not encoded within the c-*myc* gene (*7*). The specific immunoprecipitation of the 49K protein with the anti(c-myc 5′ exon), however, suggests that this protein is of interest as a c-myc–related protein. The 49K c-myc–related protein was therefore analyzed in parallel with the $p65K^{c\text{-}myc}$ protein. The anti(c-myc 5′ exon) was used for the 49K protein, whereas the anti(c-myc N-ter) was used for the $p65K^{c\text{-}myc}$ protein.

Cells labeled with [^{35}S]methionine were fractionated in order to determine the subcellular location of the $p65K^{c\text{-}myc}$ protein. The cells were harvested and gently disrupted in a hypotonic buffer (Dounce homogenizer), and the cell cytoplasm was further fractionated on two "cushions" of sucrose. Membranes visible at the interface of the two sucrose cushions were collected as well as the upper two-thirds of the gradient containing soluble cytoplasmic proteins. The latter fraction is referred to as the cytosol. Cell nuclei were washed once in isotonic buffer and disrupted by sonication in a buffer containing a mixture of detergents. Equal amounts of cell equivalents from each subcellular fraction were used for immunoprecipitation. This analysis showed that about 90 percent of the $p65K^{c\text{-}myc}$ protein was associated with the nuclear fraction (Fig. 1A). The remaining 10 percent was recovered in the membrane fraction, whereas the $p65K^{c\text{-}myc}$ protein was undetectable in the cytosol fraction. In contrast, the 49K c-myc related protein was found almost exclusively in the cytosol fraction with only a small percentage of the protein recovered in the nuclear fraction (Fig. 1B).

The experiment presented in Fig. 1 was performed with HeLa cells, but identical results were obtained with human promyelocytic leukemia (HL60) cells or Burkitt lymphoma cells. In order to control for nonspecific association of the $p65K^{c\text{-}myc}$ protein with the nuclear fraction, the subcellular fractionation was performed in a number of different ways including detergent lysis or the use of spermine and spermidine to stabilize the cell nuclei. In all cases most of the $p65K^{c\text{-}myc}$ protein fractionated with the cell nuclei whereas the 49K c-myc–related protein was always recovered in the cytosol fraction. Furthermore, nuclei isolated from HL60 cells extracted by different procedures showed that most of the $p65K^{c\text{-}myc}$ protein required a combination of high salt and detergents in order to be efficiently solubilized, suggesting a tight association with the cell nucleus (data not shown).

The MC29 viral transforming polyprotein $p110K^{gag\text{-}myc}$ binds to double-stranded DNA (*5*). Experiments were therefore performed in order to determine whether the human $p65K^{c\text{-}myc}$ protein is also a DNA binding protein. The DNA binding was performed as an affinity chromatography as described by Prives *et al.* (*9*) for the SV40 large tumor antigen. Calf thymus double-stranded DNA was sheared to a size of approximately 20 kilobases and attached to washed cellulose. A nuclear extract from HL60 cells labeled with [^{35}S]methionine was solubilized in a high salt buffer containing a mixture of detergents. The solubilized sample was then diluted 20-fold in a DNA binding buffer adjusted to *p*H 6.0 and chromatographed on a column containing double-stranded DNA. The column was extensively washed until no radioactivity was recovered and proteins bound to the column were eluted with a high salt buffer adjusted to *p*H 8.5. An equal amount of cell equivalents from the flow-through and the eluted fractions were immunoprecipitated with different antisera to c-myc. The $p65K^{c\text{-}myc}$ protein was predominantly found in the fractions eluted from the column (Fig. 2). In contrast, the 49K c-myc–related protein was exclusively found in the flow-through fractions, indicating that this protein had no affinity for double-stranded DNA in this assay. Chromatography on agarose columns containing (single-stranded DNA) showed that the $p65K^{c\text{-}myc}$ protein also had a high affinity for calf thymus

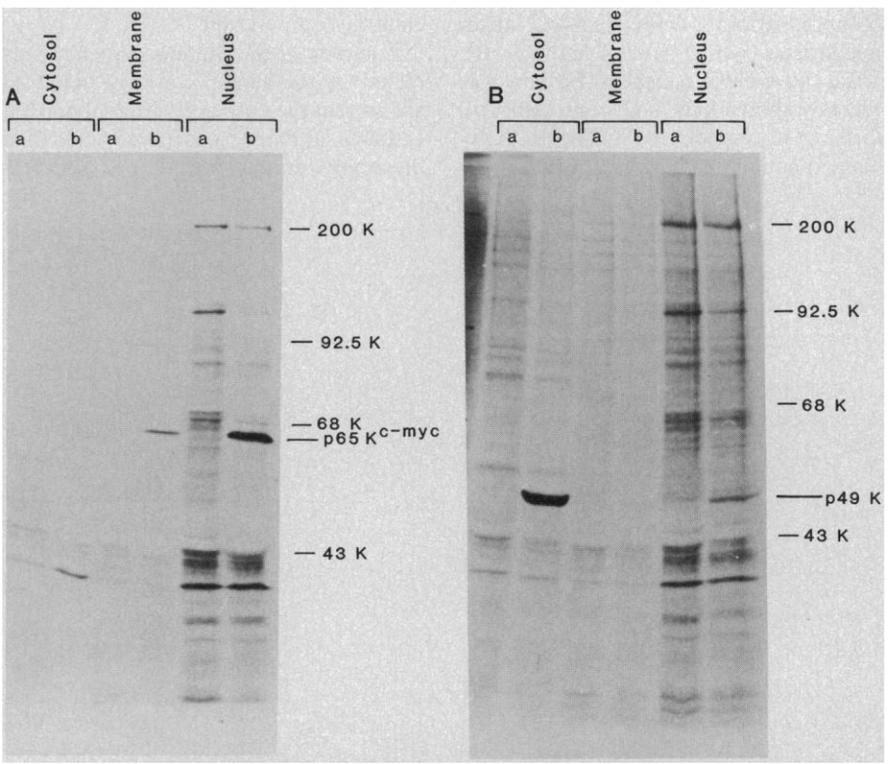

Fig. 1. Immunoprecipitation of subcellular fractions with antisera to human c-myc protein. HeLa cells (2×10^8) were labeled with [^{35}S]methionine (50 μCi/ml) for 15 hours in RPMI 1640 medium containing fetal calf serum (10 percent) and 1/20 the normal amount of methionine. Cells were washed once in ice-cold phosphate-buffered saline (PBS) and resuspended (5×10^7 cells per milliliter) in 0.02*M* Hepes *p*H buffer, 7.8, 1 m*M* $MgCl_2$, 0.5 m*M* $CaCl_2$, 1 m*M* dithiotreitol, and 1 m*M* phenylmethylsulfonyl fluoride (PMSF). The cells were incubated for 10 minutes on ice and then disrupted by 12 strokes with a tight-fitting Dounce homogenizer and centrifuged at 800*g* for 2 minutes. The sediment, which contained the nuclear fraction, was washed once in an isotonic buffer at *p*H 7.5 and resuspended by brief sonication at 10^8 cell equivalents per milliliter in RIPA buffer [0.05*M* tris-HCl, *p*H 7.5, 0.5*M* NaCl, 0.1 percent sodium dodecyl sulfate (SDS), 0.5 percent sodium deoxycholate, 1 percent Triton X-100, and 1 m*M* PMSF]. The supernatant was layered on a gradient consisting of 3 ml of 80 percent sucrose and 5 ml of 20 percent sucrose and centrifuged for 90 minutes at 35,000 rev/min (SW40 rotor). The upper two-thirds of the gradient was removed and contained the cytosol fraction. The interphase contained the membrane fraction, which was collected and diluted with two volumes of RIPA buffer. An equal amount of cell equivalents from each sample was diluted with ten volumes of PBS containing 1 percent Triton X-100 and 1 m*M* PMSF, and immunoprecipitated with the serum indicated. The immunoprecipitates were washed five times in RIPA buffer and analyzed on 10 percent SDS-PAGE (polyacrylamide gel electrophoresis) (*17*). Fractions used for immunoprecipitation are indicated at the top of the figures. (A) Immunoprecipitation with (a) serum from a nonimmunized rabbit (NRS) or (b) with anti(c-myc N-ter). (B) Immunoprecipitation (a) with NRS or (b) with anti(c-myc 5′ exon).

single-stranded DNA (data not shown).

Our data show that the p65k$^{c\text{-}myc}$ protein is a nuclear DNA binding protein (Figs. 1 and 2). These results correlate with the findings that the MC29 viral myc polyprotein is a nuclear DNA binding protein (*5*). Two other members of the myelocytomatosis virus family, OK10 and MH2, generate v-myc proteins, 61K to 63K in size, unlinked to any other viral antigen through the activity of a subgenomic v-*myc* messenger RNA (*6*). These v-myc proteins are also nuclear, but a DNA binding property remains to be established.

The nuclear localization and DNA binding property of the c-myc protein suggests that the c-*myc* oncogene has a function distinct from most other oncogene proteins thus far identified. This difference may even include oncogenes harbored by other defective avian leukemia viruses (v-*myb*, v-*erb*A and v-*erb*B), since the v-*erb*B oncogene protein was recently shown to be a glycoprotein (*10*). Cell transformation assays suggest, however, functional similarities between the adenovirus Ela proteins and the c-myc protein (*11*). Accordingly, the adenovirus Ela proteins are found in the cell nucleus (*12*), but no binding to DNA has yet been demonstrated. It, therefore, appears likely that the DNA binding property of the c-myc protein creates an additional function for the c-myc protein not associated with the Ela proteins.

Analysis of messenger RNA isolated from several different tissues showed that the c-*myc* messenger RNA is expressed in various tissues and cell types (*13*). Consistent with this, we were able to immunoprecipitate the p65K$^{c\text{-}myc}$ protein from a large number of different cell types (*7*). The wide distribution of the c-myc protein suggests an important function that is required for every type of cell. Our results suggest that this function is a nuclear event that involves binding to cellular DNA.

A survey of the protein sequence predicted for the human c-myc protein (*14*) shows that the carboxyl terminal portion contains far more basic amino acids than the amino terminal part. A common feature of many DNA binding proteins is the presence of a DNA binding domain rich in basic amino acids that, in combination with one or several other domains, create a multifunctional protein (*15*). The sequence of the human c-myc protein suggests that the carboxyl terminal part represents the DNA binding domain, whereas the amino terminal domain has another yet to be identified function. The conservation of the DNA binding property between the v-myc and c-myc protein suggests that the DNA binding capability is of functional importance for the c-myc protein. Accordingly, the greatest amount of amino acid homology between the v-myc and c-myc proteins is found in the carboxyl terminal portion of the two proteins (*14*). The p65K$^{c\text{-}myc}$ protein showed high affinity for double- or single-stranded DNA as measured by DNA affinity chromatography. A binding with no apparent sequence specificity of a protein to DNA as in the case of the adenovirus single-stranded 72K DNA binding protein or the gene 32 protein of bacteriophage T4 (*16*) may reflect a structural rather than a more specific—for example, a regulatory—role for the protein. The low levels of the p65K$^{c\text{-}myc}$ protein and its wide distribution in different cell types suggests a more specific function for the c-myc protein that is connected with its DNA binding capacity. It is, for example, possible that the c-myc protein binds preferentially to a specific DNA sequence. The availability of a specific antiserum to the c-myc protein makes it possible to study specific DNA interactions in more detail. The greatly enhanced expression of the c-myc protein after activation of cells with mitogens (*7*) suggests that DNA binding capability may correlate with cell proliferation.

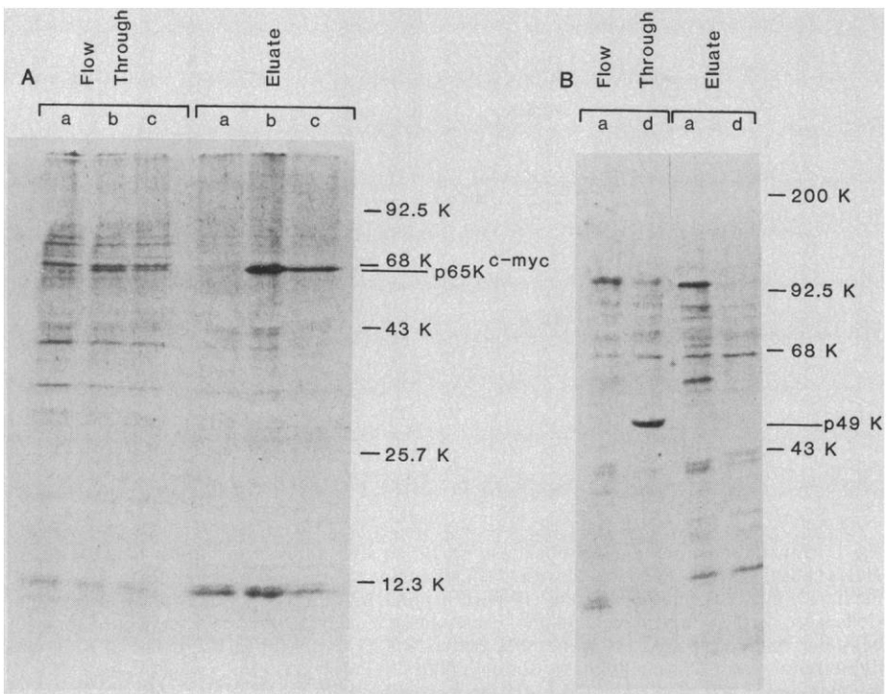

Fig. 2. DNA binding assay. A nuclear extract prepared from [^{35}S]methionine labeled HL60 cells (see legend to Fig. 1) was solubilized in RIPA buffer containing 2*M* NaCl. The sample was solubilized and diluted to 1/20 its original volume in a DNA binding buffer (0.02*M* sodium phosphate, *p*H 6.0, 0.14*M* NaCl, 0.5 percent Nonidet P40, and 0.2 m*M* PMSF). The sample was applied to a column containing calf thymus double-stranded DNA and cellulose that had been equilibrated with the DNA binding buffer. The column was washed extensively with the DNA binding buffer and proteins bound to the column were eluted with 0.05*M* tris-HCl, *p*H 8.5, 1*M* NaCl, 0.5 percent Nonidet P40, and 0.2 m*M* PMSF. Samples for immunoprecipitation were diluted with two volumes of PBS, 1 percent Triton X-100, and 1 m*M* PMSF and an equal amount of cell equivalents from the flow-through or the eluted fractions were immunoprecipitated with the sera indicated. The immunoprecipitates were analyzed with 13 percent SDS-PAGE (A) or with 10 percent SDS-PAGE (B). (a) Serum from a nonimmunized rabbit; (b) anti(c-myc N-ter); (c) anti(c-myc N-ter) first incubated with 80 μg of the synthetic human c-myc peptide; (d) anti(c-myc 5′ exon). Indicated at the top of the figures are the fractions used for immunoprecipitation.

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A Cell Line Expressing Vesicular Stomatitis Virus Glycoprotein Fuses at Low *p*H

Abstract. *A stable cell line expressing a complementary DNA clone encoding the vesicular stomatitis virus glycoprotein fused and formed polykaryons at* p*H 5.5. The formation of polykaryons was dependent on the presence of glycoprotein anchored at the cell surface and could be prevented by incubation of cells with a monoclonal antibody to the glycoprotein. Fusion occurred at a* p*H 0.5 unit lower than that observed for cells infected with vesicular stomatitis virus.*

A virus particle must enter the host cell to grow. There are two ways in which enveloped viruses are known to enter the cell (*1*). Paramyxoviruses such as Sendai virus can enter through direct fusion (in a *p*H-independent manner) of the virion envelope with the plasma membrane of the cell (*1, 2*). The second path of entry, which is used by influenza virus (*1, 3*), Semliki Forest virus (SFV) (*1, 3, 4*), and vesicular stomatitis virus (VSV) (*1, 3, 5, 6*), is the internalization of virus particles in coated vesicles. The internalized vesicle is acidified, possibly after fusing with other intracellular vesicles (*1, 3*). The low *p*H in the vesicles containing virus particles causes fusion of the viral envelope with the membrane of that vesicle (*3*), and the viral nucleocapsids are released into the cytoplasm.

Direct evidence for a membrane fusion activity of viral glycoproteins has been obtained by expressing cloned complementary DNA's (cDNA's) encoding the SFV glycoproteins E1 and E2 (*7*) and the influenza hemagglutinin glycoproteins HA1 and HA2 (*8*). In each case the respective glycoproteins, when expressed transiently in eukaryotic cells, were shown to promote cell-to-cell fusion at low *p*H. It is believed that the hydrophobic amino terminus of HA2 of influenza virus is required to promote membrane fusion (*8*). The E2 protein of SFV does not promote fusion alone, but when both E1 and E2 are present on the cell surface, fusion will occur at low *p*H (*7, 9*). It has been suggested that a hydrophobic amino acid sequence near the amino terminus of E1 might play a crucial role in fusion (*7, 9*).

The VSV glycoprotein (G protein) is a single polypeptide chain that is held in the viral membrane by a domain of hydrophobic amino acids near the carboxyl terminus (*10*). More than 95 percent of each protein molecule is exposed on the surface of the virion. It has been observed that VSV-infected cells will fuse at low *p*H and that virus particles alone will promote cell-to-cell fusion at low *p*H (*1*). Cell fusion was thought to be mediated by G protein at the cell surface.

In the study reported here we attempted to determine whether G protein, in the absence of other VSV proteins, will promote cell fusion at low *p*H. We previously described a mouse cell line (CG1) that expresses VSV G protein at the cell surface (*11*). These cells are ideal for investigating the role of G protein in cell fusion because they express this protein in the absence of other viral proteins, for example the VSV matrix protein, which could affect fusion by interacting with G protein. The matrix protein may interact with the cytoplasmic domain of G protein during virus maturation (*12*). Stable expression of G protein in CG1 cells has been established with a hybrid expression vector that includes the SV40 early promoter (*13*), cDNA sequences encoding normal G protein (*10*), the SV40 small t intron, SV40 early polyadenylation signals (*13*), and the 69 percent subgenomic DNA transforming fragment

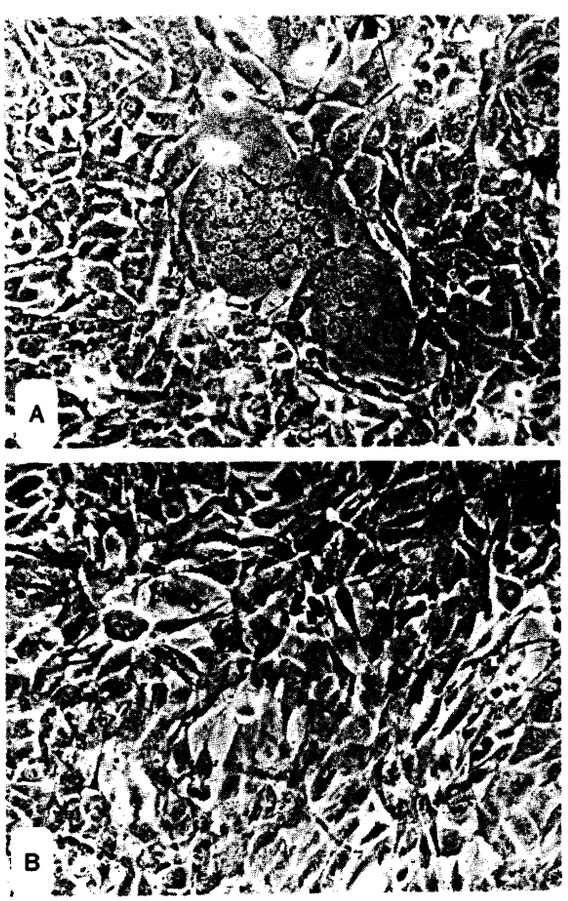

Fig. 1. Formation of polykaryons as a result of cell fusion at low *p*H. CG1 or CTG1 cells (1×10^6) were plated in 50-mm tissue culture dishes. The cells were grown for 16 hours in Dulbecco's modified Eagle's medium (DMEM) plus 5 percent fetal bovine serum in an atmosphere containing 10 percent CO_2. The culture medium was removed and replaced with 2 ml of prewarmed (37°C) fusion medium (1.85 m*M* $NaH_2PO_4 \cdot H_2O$, 8.39 m*M* $NaHPO_4 \cdot 7H_2O$, 2.5 m*M* NaCl), 10 m*M* Hepes, and 10 m*M* 2-(*N*-mopholino)ethanesulfonic acid). This buffer was adjusted to a final *p*H of 5.25. The cells were incubated in this for 1 minute; then the fusion medium was removed and replaced with DMEM plus 5 percent fetal bovine serum. In this experiment the cultures were returned to an incubator (37°C and 10 percent CO_2) and examined for fusion 12 hours later as a convenience, but it should be noted that fusion could be detected within 2 hours after incubation in fusion medium. (A) Polykaryon formation in CG1 cells. (B) Typical field of CTG1 cells, showing the absence of polykaryon formation. The photographs were taken with Polaroid film (type 55) and an Olympus inverted microscope.