Fig. 2 Kinetics of GTP hydrolysis under the conditions of Fig. 1. (●) Unsonicated sample; (○) 3 s; (■) 9 s sonicated samples. (♦) and (□), respectively, show the non-significant GTPase activity exhibited by the boiled MAPs and the phosphocellulose purified tubulin.

the frequency of exchange of tubulin subunits at the ends of the microtubules. Knowledge of such a relationship between GTPase activity of tubulin and the polymerisation reaction should permit us to investigate the mechanism and the regulation of tubulin polymerisation *in vitro* and *in vivo*.

For example, the rapidity of subunit exchange at the polymerisation equilibrium should be related to the dynamic stability of the microtubules, and the steady-state rate of GTP hydrolysis should also reflect this dynamic stability. It has been reported⁹ that MAPs stabilise microtubules by slowing down the rate of microtubule depolymerisation. Also, the critical concentration for polymerisation in the presence of Mg^{2+} (ref. 7) is somewhat higher than that in the presence of MAPs². We found that the GTPase activity of solutions of microtubules which polymerised in the presence of Mg^{2+} and glycerol¹ was three times higher than that of solutions of microtubules polymerised in the presence of MAPs. According to our model, and with equal length distribution, this would also indicate a greater stability of MAP-polymerised microtubules as compared to microtubules polymerised in the presence of Mg^{2+} and glycerol. Finally, according to the model proposed, the suggestion that the rate of GTP hydrolysis should be directly proportional to the concentration of microtubule ends is valid only at the polymerisation equilibrium. The observed redistribution of microtubule length after sonication, however, shows that, in these conditions, the number and size of polymers are not yet at equilibrium even if the concentration of dispersed monomers retains approximately its equilibrium value. This is a situation of approaching equilibrium, that is, the rate of polymerisation is nearly equal to the rate of depolymerisation. This situation has been analysed by Oosawa¹⁰; in our case, however, the difference in rate must be very small and so does not alter our conclusion.

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5-Bromodeoxyuridine induces expression of a tumour-specific surface antigen on normal avian cells

TUMOUR specific surface antigen (TSSA) has been described on all cell types transformed *in vivo* or *in vitro* by avian sarcoma virus (ASV)^{1,2}. It is distinct from the viral structural antigens and seems to be expressed only on morphologically transformed cells. However, it can also be present exceptionally on untransformed cells infected at non-permissive temperature with ASV ts mutants³. We present here evidence that treatment of normal chick embryo fibroblasts (CEF) and Japanese quail embryo fibroblasts (JQEF) with 5-bromodeoxyuridine (BRdU) induces a new surface antigen which reacts with rabbit serum to ASV-transformed rat XC cells. This antigen is related to TSSA induced on both ASV-transformed mammalian cells and CEF.

The induction of a cell-surface antigen related to TSSA was studied on embryonic fibroblasts of various avian species treated for 15 d with BRdU ($4 \mu\text{g ml}^{-1}$) and they were compared with CEF and mammalian fibroblasts transformed by Rous sarcoma virus (RSV). TSSA expression was measured by a mixed haemadsorption test⁴ which is specific for ASV-induced TSSA^{5,6}. As Table 1 shows, this test was positive with both CEF productively infected with different RSV strains and with non-permissive rat and hamster cells transformed by the same strains, whether or not viral antigens were expressed at their surface. Moreover, the test was negative with CEF productively infected with the helper virus RAV1 and with hamster cells transformed by adenovirus 12 or by chemical carcinogens (data not shown).

Cells were seeded sparsely on coverslips and incubated with TSSA-specific anti-XC cell rabbit serum, and then with sheep red blood cells (SRBC) coated with rabbit anti-SRBC serum and goat anti-7S rabbit globulin. Both CEF and JQEF, after 2 weeks in the presence of BRdU, expressed a surface antigen cross-reacting with anti-XC serum, whereas duck embryo fibroblasts (DEF) remained negative (Table 2), and BRdU-treated cells expressed less antigen than ASV-transformed CEF and mammalian cells.

The difference in quantitative expression of the antigen could have resulted if the TSSA expressed on ASV-transformed cells were not a single antigen, but a complex consisting of at least two antigens. In that case, the antigen expressed on BRdU-treated cells could have consisted of only one of the determinants. To check this possibility, extensive cross-absorption experiments were performed. The results (Table 3) suggest that there are at least two antigenic determinants of ASV-induced TSSA, one common to ASV-transformed CEF and mammalian cells and BRdU-treated CEF, and the other specific to ASV-transformed cells.

A possible re-expression of embryonic antigens able to cross-react with anti-XC serum in both BRdU-treated CEF and ASV-transformed cells was investigated by extensive absorption of rabbit anti-XC serum with a homogenate of 10-d-old chick embryos followed by testing of the absorbed serum against BRdU-treated CEF and ASV-transformed

Table 1 Comparative expression of TSSA and viral proteins on different transformed cell lines

Cells	Virus	Subgroup	Cell phenotype ¹	TSSA	Membrane expression of gp85	p prot.
CEF	SR-RSV	A, D	T	+	+	+
CEF	B-RSV (RAVI)	A	T	+	+	+
CEF	PR-RSV	C	T	+	+	+
CEF	RAVI	A	NT	—	+	+
Rat XC	PR-RSV	C	T	+	+	+
Rat TWERC	PR-RSV	C	T	+	—	—
Hamster RS2	SR-RSV	D	T	+	+	+
RB 12/5	B-RSV (RAVI)	A	T	+	—	—

T, transformed; NT, not transformed.

*Including p27.

†Other than p27 (compare ref. 7).

cells (Table 3). No diminution in the positive reaction was observed with any cell type. However, this experiment did not rule out the possibility that the antigen induced by BRdU and ASV-induced transformation is a very early embryonic antigen. Experiments are in progress to test this possibility.

So far, TSSA has been considered a specific marker for ASV-induced cell transformation, because all ASV-transformed cells, either avian or mammalian, express TSSA. Moreover, quail cells transformed by murine sarcoma virus do not express TSSA⁸. But here we report that incubation of normal CEF with BRdU induces the expression of at least one determinant of TSSA in the absence of ASV. As already noted^{9,10}, BRdU elicits changes in cell morphology consisting of an enlargement and flattening of the cytoplasm, which are clearly different from transformation by ASV in which the cells become rounder, detached from neighbouring cells and able to grow in three dimensions. However, BRdU elicits an increase of cellular agglutinability by concanavalin A comparable with that observed after ASV-induced transformation¹¹. Thus the appearance on BRdU-treated cells of an antigen related to a deter-

minant of TSSA together with enhanced lectin agglutinability may be considered a BRdU-induced step towards transformation.

We investigated whether the expression of TSSA in BRdU-treated cells could be related to some modification of the expression of the endogenous virus of CEF, notably expression of the viral p proteins which can appear at the cell surface¹². Indeed, endogenous type C viruses have been induced efficiently by BRdU in growing mouse embryo fibroblasts¹³, and in CEF the production of inducible virus seemed to be a unique characteristic of some but not all pedigrees of CEF¹⁴. In the CEF used in our investigation no virus was induced after BRdU treatment. However, immunofluorescence tests on fixed cells with a monospecific

Table 2 Expression of TSSA on BRdU-treated avian and ASV-infected chicken or mammalian cells

Cells	Normal rabbit serum	Rabbit anti-XC ⁺ serum		
		1/20	1/40	1/80
CE-BL	—	—	—	—
CE-BL + BRdU	—	+++	+++	+
CE-C	—	—	—	—
CE-C + BRdU	—	+++	+++	+
CE-BL + ASV	—	+++	+++	+
JQEF	—	—	—	—
JQEF + BRdU	—	+++	+++	—
DEF	—	—	—	—
DEF + BRdU	—	—	—	—
BHK21/13	—	—	—	—
TWERC	—	+++	+++	+++
RBH	—	+++	+++	+++

TSSA were detected by a mixed haemadsorption test. The degree of haemadsorption was estimated as follows: + + +, test cells completely covered with indicator red blood cells; + +, cells moderately covered; +, cells covered with scattered indicator red blood cells. XC, cell line derived from a rat sarcoma produced by ASV; CE-BL, chick embryo fibroblasts from gs⁺ embryos; CE-C, chick embryo fibroblasts from gs⁻ embryos; JQEF, Japanese quail embryo cells; DEF, duck embryo cells; BHK21/13, normal hamster cell line used as a control; TWERC, non virogenic rat cell transformed by ASV; RBH, virogenic hamster cell deriving from a hamster tumour by ASV.

Table 3 Adsorption of rabbit anti-XC serum with BRdU-treated and ASV-transformed CEF

Cells	Rabbit anti-XC	Antisera	
		Rabbit anti-XC absorbed on CE-BL + BRdU	Rabbit anti-XC absorbed on CE-BL + ASV
CE-BL	—	—	—
CE-BL + BRdU	++	—	—
CE-BL + ASV	++	+	—
TWERC	+++	+++	+++
RBH	+++	+++	+++

Residual TSSA activity was measured using the technique described in Table 2, after absorption on BRdU-treated or ASV-transformed CEF. For details of cells see Table 2.

hyperimmune serum against the viral p27 protein revealed increased expression of this protein in the BRdU-treated CEF (Table 4), whereas no expression of p27 was observed in BRdU-treated JQEF and DEF which do not carry the type-specific antigen of subgroup E of ALV (chf). Enhancement of the expression of gs antigen(s) has also been noted in virogenic ASV-transformed mammalian cells¹⁵.

Our results show that treatment of normal CEF and JQEF with BRdU elicits the appearance of an antigen related to ASV-specific TSSA expressed on ASV-transformed chicken and mammalian cells. They further suggest that ASV-specific TSSA is a complex of at least two antigens, one of which is related to the BRdU-induced antigen. The nature of the second antigen and its significance remain to be determined. Two models at least can be proposed. First, because normal CEF and JQEF carry a gene closely related, in molecule hybridisation experiments,

Table 4 Expression of viral p27 protein on BRdU-treated and normal CEF, JQEF and DEF

Cells	Degree of fluorescence		Degree of fluorescence		Degree of fluorescence	
	100%+++	50%+++	100%++	50%++	100%+	50%+
CE-BL	20	20	40	80	80	160
CE-BL+BRdU	40	160	80	640	1280	2560
CE-C	20	20	20	20	40	80
CE-C+BRdU	20	40	640	1280	2560	2560
JQEF	20	20	20	20	20	20
JQEF+BRdU	20	20	20	20	20	20
DEF	20	20	20	20	20	20
DEF+BRdU	20	20	20	20	20	20

The figures are the reciprocal of the serum dilution giving the indicated degree of fluorescence in 100% or 50% of the cells. Cells were seeded sparsely on coverslips in Leighton tubes which were kept at 37 °C. One day later they were washed with phosphate-buffered saline, fixed with acetone for 10 min, incubated for 45 min at 37 °C with increasing dilutions of the monospecific rabbit anti-p27 ASV immune serum and incubated for 30 min at room temperature with fluorescein conjugated globulin anti-rabbit 7S IgG. + + +, Intense continuous fluorescence; + +, intense granular fluorescence; +, moderate granular fluorescence. For details of cells see Table 2.

to the viral *src* gene responsible for cell transformation¹⁸, the BRdU-induced TSSA could be the protein coded by the cellular *src* (sarc) gene. In this case, BRdU could induce the expression of the protein, which is normally repressed in untreated cells. The lack of reactivity of the anti-XC antiserum with BRdU-treated DEF could be due to the antigenic unrelatedness of the products of endogenous *src* of DEF and CEF. Experiments using specific antisera to BRdU-treated and untreated DEF are in progress to test this hypothesis. Second, the TSSA-related antigen(s) expressed on BRdU-treated CEF and JQEF could be unrelated to the antigenic determinant coded by the viral *src* gene and related to another cellular determinant of ASV-specific TSSA. This determinant could be a cross-reacting antigen common to avian and mammalian cells and expressed after both BRdU treatment and virus-induced transformation—for instance, an early embryonal antigen. Because BRdU treatment also enhanced the expression of p proteins in treated CEF, the mechanism of induction of the TSSA-related antigen might also be the same as that of induction of the cryptic chick helper factor.

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Errata

In the letter by R. S. Bradley and J. England, *Nature* **271**, p. 736, the title should read: Influence of volcanic dust on glacier mass balance at high latitudes. In paragraph 4, line 4, (TxMDD) should read (TxMDD). In paragraph 5 the equations in lines 7 and 8 should read: $y = -2.79x + 224.8$ and $r = -0.94$. In line 26 for 1933–64 read 1963–64. In paragraph 7, for $P < 0.01$ read $P < 0.001$. Figure 4 legend should include an extra line: Asterisks indicate data for ≤ 1 month estimated.

In the letter 'Gating properties of acetylcholine receptor in newly formed neuromuscular synapses' by H. R. Brenner & B. Sakmann *Nature* **271**, p. 366, the time calibration bar in Fig. 2 should be marked 20 ms, and that in Fig. 3 should read 2 ms.

In the letter 'Regeneration of endoderm by ectoderm isolated from mouse blastocytes' by R. Pederson, *Nature* **270**, 435, in paragraph 2 line 3 for 'thin inner cell masses' read 'then inner cell masses'.

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