Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7122–7126, November 1984
Cell Biology

src- and *fps*-containing avian sarcoma viruses transform chicken erythroid cells

PATRICIA KAHN, BECKY ADKINS, HARTMUT BEUG, AND THOMAS GRAF

European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, Federal Republic of Germany

Communicated by Peter K. Vogt, July 23, 1984

ABSTRACT **We report here that several oncogene-transducing avian sarcoma virus strains, namely Rous sarcoma virus (*src*), Fujinami sarcoma virus (*fps*), and PRCII (*fps*), transform avian erythroid cells *in vitro* and *in vivo*. The *src*- and *fps*-transformed erythroblasts grow *in vitro* for 20–30 generations, require special growth conditions, and tend to differentiate spontaneously. In these properties, they resemble erythroid cells transformed with the *erbB*-containing H strain of avian erythroblastosis virus (AEV-H) but differ from those transformed with AEV-ES4 (*erbA*, *erbB*), which grow under standard culture conditions and rarely differentiate spontaneously. Erythroblasts transformed with viruses carrying temperature-sensitive mutations in the *src* or *fps* oncogene and then shifted to the nonpermissive temperature in the presence of anemic serum (as a source of an erythropoietin-like factor) differentiate terminally into erythrocytes. These results demonstrate that several members of the *src* gene family other than *erbB* have the capacity to transform erythroid cells.**

The avian sarcoma viruses (ASVs) and defective leukemia viruses (DLVs) are acutely transforming retroviruses, which contain cell-derived oncogenic sequences. The DLVs transform predominantly hematopoietic cells, with most strains showing specificity for cells within one lineage; some strains also induce sarcomas *in vivo* and transform fibroblasts in culture (1). In contrast, the ASVs cause almost exclusively fibrosarcomas and transform predominantly fibroblasts *in vitro*, although the Rous sarcoma virus (RSV) is also able to transform certain types of mesenchymal and epithelial cells (2). None of the ASVs has been shown to transform avian hematopoietic cells.

However, several recent findings led us to reexamine whether ASVs can transform chicken bone marrow cells. First, the erbB protein, which is responsible for the erythroid transforming capacity of the avian erythroblastosis virus (AEV) (3, 4) was found to contain a region with ≈40% sequence homology to the kinase domain of the RSV src protein (5), which in turn is closely homologous to the fps transforming protein of Fujinami sarcoma virus (FSV) (6) and to the yes protein of Y73 (7). Second, the viral *src* gene was shown to stimulate splenic colony formation in mice (8), although cells from these colonies could not be propagated *in vitro*, and it is therefore not clear to what hematopoietic lineage they belong. Lastly, conditions for culturing avian hematopoietic precursors and certain DLV-transformed cells have been significantly improved in the last few years (9, 10). Our results indicate that viruses that carry the *src* or *fps* oncogene, and probably also those with the *yes* oncogene, are able to transform chicken erythroid cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Viruses. The following ASV strains, with the helper virus shown in parentheses, were used: the Schmidt–Ruppin D RSV strain and its temperature-sensitive (ts) mutant NY68RSV, which was derived from Schmidt–Ruppin subgroup A (11); PRCII (PRCII AV); FSV (FAV) and its ts mutant NY225FSV (FAV) (12); and Y73 (RAV-1). AEV-ES4 (RAV-2), the ts mutant 167AEV (RAV-2) (13), AEV-H (RAV-1) (14), and the preparation and titration of virus stocks have been described (15).

Cell Culture. All cells were derived from our SPAFAS chicken flock maintained in Heidelberg. Unless otherwise indicated, cells were grown in Dulbecco's modified Eagle's medium (DME medium) supplemented with 8% fetal calf serum/2% inactivated chicken serum (all from GIBCO)/10 mM Hepes, pH 7.3, at 37°C in 5% CO_2/95% air. Virus-transformed erythroid cells were grown in CFU-E medium (16) with insulin (1 μg/ml) (Actrapid; Novo Industrie, Mainz, FRG) in 2% CO_2. The preparation of methylcellulose and of CFU-M medium have been described (10, 15). For differentiation assays, 1×10^6 cells per ml were seeded into CFU-E medium supplemented with 5% anemic chicken serum as a source of erythropoietin-like activity (17). Erythropoietin dependence was tested by replacing the anemic serum with an equal amount of a pretested normal chicken serum, which did not contain detectable erythropoietin-like activity.

***In Vitro* Transformation of Bone Marrow Cells.** Bone marrow cells were infected and then seeded in methylcellulose-containing medium supplemented with 1% dimethyl sulfoxide and 5% of a selected chicken serum batch, as described (15). Alternatively, "mass-transformed" (i.e., nonclonal) populations were obtained in liquid culture as described by Graf (18).

Assays for Differentiation Markers. Hemoglobin was detected by benzidine staining at acid or at neutral pH (17). The former procedure is more sensitive and therefore detects lower levels of hemoglobin than the latter (13). Rabbit antiserum against the avian erythroid-specific marker histone H5 (19), rabbit anti-erythroblast and anti-reticulocyte/erythrocyte antisera (19), and the myelomonocytic cell-specific monoclonal antibody MC51-2 (20), were used in indirect immunofluorescence assays as described (19).

Detection of Transforming Proteins. Erythroblasts suspended at 5×10^6 cells per ml and adherent fibroblasts were lysed in radioimmunoprecipitation (RIP) buffer (21). Lysates were immunoprecipitated (21) with an excess of tumor-bearing rabbit serum (22) or of affinity-purified rabbit anti-gag antibody (23). The protein kinase activity present in immune complexes was assayed as described (21). The reaction mixtures were then run on $NaDodSO_4$/12.5% polyacrylamide gels (24). Processing of the gels for fluorography and exposure to Kodak XAR film were carried out as described (23).

Infection of Experimental Animals and Isolation of *in Vivo*-Transformed Cells. Newborn chicks were injected i.v. at the knee joint with $\approx 10^5$ focus-forming units. Blood smears from the injected animals were examined 3 times a week for the presence of blast-like cells (17). Cells were recovered from the peripheral blood of positive animals and plated in culture as described (17).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ASV, avian sarcoma virus; DLV, defective leukemia virus; RSV, Rous sarcoma virus; AEV, avian erythroblastosis virus; FSV, Fujinami sarcoma virus; ts, temperature sensitive; wt, wild type.

Table 1. Fibroblast and bone marrow transforming activities of ASVs

Virus group	Strain	Oncogene	FFU/ml on fibroblasts	CFU/ml on bone marrow cells
ASVs	RSV	*src*	5×10^5	50
	FSV	*fps*	3×10^5	80
	PRCII	*fps*	5×10^5	10
	Y73	*yes*	5×10^6	2*
DLVs	AEV-ES4	*erbA, erbB*	5×10^5	2000
	AEV-H	*erbB*	1×10^5	700

FFU, focus-forming units; CFU, colony-forming units.
*Small colonies that did not grow after being picked from methylcellulose-containing medium.

RESULTS

ASVs Transform Chicken Bone Marrow Cells *in Vitro*. To determine whether ASVs can transform hematopoietic cells *in vitro*, bone marrow cells from 1- to 3-week-old chickens were infected with various dilutions of high titer ASV or DLV stocks and then seeded into methylcellulose-containing medium under conditions suitable for the recovery of AEV-H-transformed erythroblasts. Bone marrow cells infected with RSV, FSV, PRCII, or Y73 gave rise within 10 days to transformed hematopoietic colonies containing up to several thousand cells and closely resembling those induced by the AEV strains. As shown in Table 1, however, they occurred at much lower frequencies. ASV- or AEV-H-induced colonies could be expanded in CFU-E medium [a medium that supports the growth of certain DLV-transformed erythroid cells (16)], where they grew with an average doubling time of 18–24 hr for 20–30 population doublings and then underwent senescence. In standard medium, these cells died within 3 days, while AEV-ES4-transformed cells were able to grow under these conditions. Y73-induced colonies could not be expanded in either standard or CFU-E medium, suggesting that they may have yet different growth requirements.

ASV-Transformed Bone Marrow Cells Represent Immature Spontaneously Differentiating Erythroid Cells. ASV-transformed bone marrow cells were compared to erythroblasts transformed by AEV-ES4 or AEV-H and to myeloblasts transformed by avian myeloblastosis virus with respect to their expression of several differentiation markers specific for the erythroid or myeloid lineage. The results (summarized in Table 2) show that the ASV-transformed cells expressed four erythroid-specific markers, including two (hemoglobin and the reticulocyte–erythrocyte cell surface antigen) that are typical of later stages of erythroid differentiation but were negative for expression of a myeloid-specific marker. As illustrated in Fig. 1, the proportion of cells expressing hemoglobin varied over a wide range between different clones. (Similarly, expression of the reticulocyte–erythrocyte cell surface antigen showed clonal variation; data not shown). Taken together, these results indicate that ASV- and AEV-H-transformed bone marrow cells belong to the erythroid lineage and that they have the capacity to differentiate spontaneously. In contrast, AEV-ES4-transformed erythroblasts (included as a control) consisted exclusively of immature cells, which did not undergo spontaneous differentiation.

Table 2. Differentiation markers expressed by transformed cells

Marker	% marker-positive cells in populations* transformed by: RSV	FSV	PRCII	AEV-ES4	AEV-H†	AMV
Histone H5	100	100	100	100	100	0
Hemoglobin (acid benzidine)	66	32	54	0.1	72	0
Erythroblast antigen	85	75	70	98	77	0
Erythrocyte antigen	85	45	65	0.1	70	0
Myelomonocytic antigen	0	0	0	0	0	98

AMV, avian myeloblastosis virus.
*Mass cultures.
†Clones (average of five to seven clones).

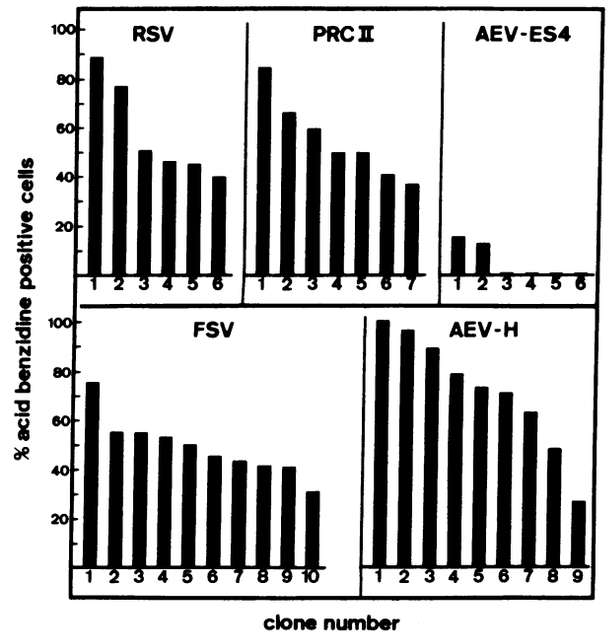

FIG. 1. Hemoglobin expression in individual clones of virus-transformed cells. Virus-transformed cell clones were stained with benzidine at acid pH. Bars indicate proportion of positive cells detected in each clone.

ASV-Transformed Erythroid Cells Express the Viral Transforming Proteins. To determine whether ASV-transformed erythroid cells produced transforming virus, supernatants from five clones each of RSV-, FSV-, and PRCII-transformed cells were tested for their ability to induce transformed foci in chicken embryo fibroblasts. Three of five clones each of FSV- and PRCII-transformed cells were nonproducers; all of the remaining clones produced virus at titers similar to, or higher than, fibroblasts transformed with the same virus stock (data not shown).

To examine whether the *src* and *fps* gene products are expressed in transformed erythroblasts, these proteins were immunoprecipitated from extracts of infected cells. The associated protein kinase activity was then assayed *in vitro*. As shown in Fig. 2, erythroid cells transformed by *src*- or *fps*-containing viruses expressed high levels of kinase activity, as did virus-transformed fibroblasts. In addition, expression of pp60*src* and p140*gag-fps* was detected after labeling of erythroblasts with [^{35}S]methionine (data not shown).

It has recently been reported that the injection of certain helper viruses into susceptible chickens can induce erythroleukemia by activating the cellular (c)-erbB gene (25). We therefore examined RSV-, FSV-, and PRCII-transformed erythroid cells for the presence of the c-erbB protein. Antiserum that recognizes both the viral p74erbB (26) and the c-erbB protein expressed by helper virus-induced erythroleukemia cells (unpublished observations) was used to immunoprecipitate [^{35}S]methionine-labeled ASV-erythroid cell extracts, which were then subjected to electrophoresis and

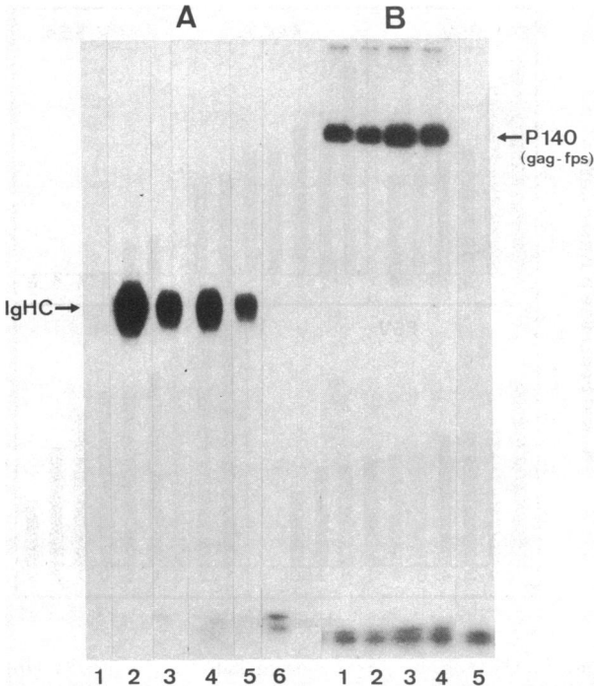

FIG. 2. Kinase activity in virus-transformed erythroblasts. (*A*) *In vitro* kinase activity of pp60*src* immunoprecipitated with tumor-bearing rabbit serum and showing phosphorylation of the IgG heavy chain (IgGHC). Lane 1, uninfected chicken embryo fibroblasts (CEFs); lane 2, RSV-transformed CEFs; lane 3, RSV-transformed erythroblasts (mass culture); lanes 4 and 5, RSV-transformed erythroblast clones a and b; lane 6, AEV-ES4-transformed erythroblast clone a. (*B*) *In vitro* autophosphorylation activity of p140gag-fps immunoprecipitated with anti-gag antibody. Lane 1, FSV-transformed CEFs; lanes 2–4, FSV-transformed erythroblast clones a, b, and c, respectively; lane 5, AEV-ES4-transformed erythroblasts (mass culture).

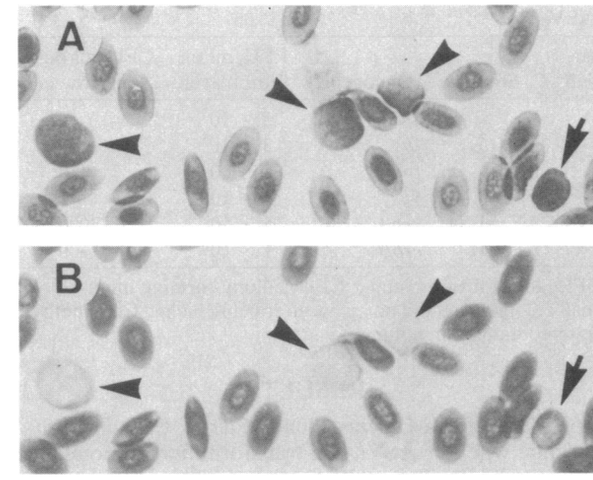

FIG. 3. Blood smears prepared from newborn chicks 10–14 days after i.v. injection of RSV were stained with benzidine at neutral pH, counterstained with Diff-Quick, and photographed under green light (*A*) and blue light (*B*), which specifically reveals hemoglobin (benzidine)-positive cells. Arrowheads indicate blast-like cells; arrows indicate erythroid cells at intermediate stages of differentiation.

autoradiography. In addition, cells were screened for the presence of cell surface erbB by indirect immunofluorescence (16). The c-erbB protein was not detected in ASV-transformed cultures by either procedure (data not shown).

ASVs Transform Chicken Erythroid Cells *in Vivo*. To determine whether ASVs are capable of transforming hematopoietic cells *in vivo*, newborn chicks were injected i.v. with RSV, FSV, PRCII, or Y73 at the knee joint, a site where these viruses induce sarcomas very inefficiently. For comparative purposes, we also injected AEV-H and AEV-ES4.

All of the viruses were lethal to the animals within 1–3 weeks of injection. While only a low percentage of the ASV-injected chicks had outwardly visible sarcomas at the time of death, autopsy revealed that ≈20% had many small internal sarcomas, often associated with the bones. In addition, many of the chicks injected with RSV, and occasionally those injected with one of the other ASVs, had hundreds of small hemorrhagic lesions lining the body cavity and extremities. Examination of blood smears revealed that all of the ASVs induced the appearance of low but significant numbers of abnormal cells in the peripheral blood of 25%–50% of the injected animals (Fig. 3). Many of these cells had an immature blast-like morphology, while others resembled erythroid cells at intermediate levels of differentiation. AEV-H induced a similar, although more severe, disease in 100% of the injected chicks. As expected from previous results (3), blood smears from animals injected with AEV-ES4 showed leukemic blasts but essentially no erythroid cells at intermediate stages of maturation.

To test whether transformed cells could be recovered from virus-infected animals, bone marrow and peripheral blood cells from RSV-, Y73-, and AEV-ES4-injected chicks showing abnormal blood smears were depleted of erythrocytes and then plated in methylcellulose-containing CFU-E or CFU-M medium. The results of this experiment are shown in Table 3. RSV- and AEV-ES4-transformed colonies were detectable within 4 days in bone marrow cultures plated under erythroid conditions but did not grow out under myeloid conditions. The peripheral blood cultures from animals injected with RSV also gave rise to transformed colonies. Five of the latter colonies expanded in CFU-E medium were found to be positive for the erythroid lineage-specific markers hemoglobin and histone H5 and for the production of transforming virus (data not shown). In contrast, the Y73 colonies picked from these cultures did not proliferate in either CFU-E or standard medium. However, they appeared to belong to the erythroid lineage, because 100% of the cells in four colonies analyzed were positive for the expression of histone H5 and a low percentage were hemoglobinized (data not shown).

Erythroid Cells Transformed with tsASV Mutants Undergo Terminal Differentiation at the Nonpermissive Temperature. We next tested whether erythroid cells transformed with ASVs carrying ts mutations in the *src* or *fps* gene would differentiate terminally into erythrocyte-like cells after shift to the restrictive temperature, as do tsAEV-ES4-transformed erythroblasts (13, 17, 27) [believed to be mutated in the *erbB* gene (16)]. Cells from mass (i.e., nonclonal) populations transformed with ts68RSV or ts225FSV or with the corresponding wild-type (wt) strains were seeded into CFU-E medium containing either normal or anemic chicken serum and then either shifted to the restrictive temperature (42°C) or maintained at 37°C. A clone of tsAEV-ES4-transformed

Table 3. Transformed erythroid colonies obtained from bone marrow and peripheral blood of virus-infected chicks

Virus	No. of chicks examined	No. of colonies per 5×10^6 cells	
		Bone marrow	Peripheral blood
RSV	3	800, 450, 800	60, 15, 80
Y73	2	250, 55	0, 0
AEV-ES4	1	40,000	15,000
None	1	0	0

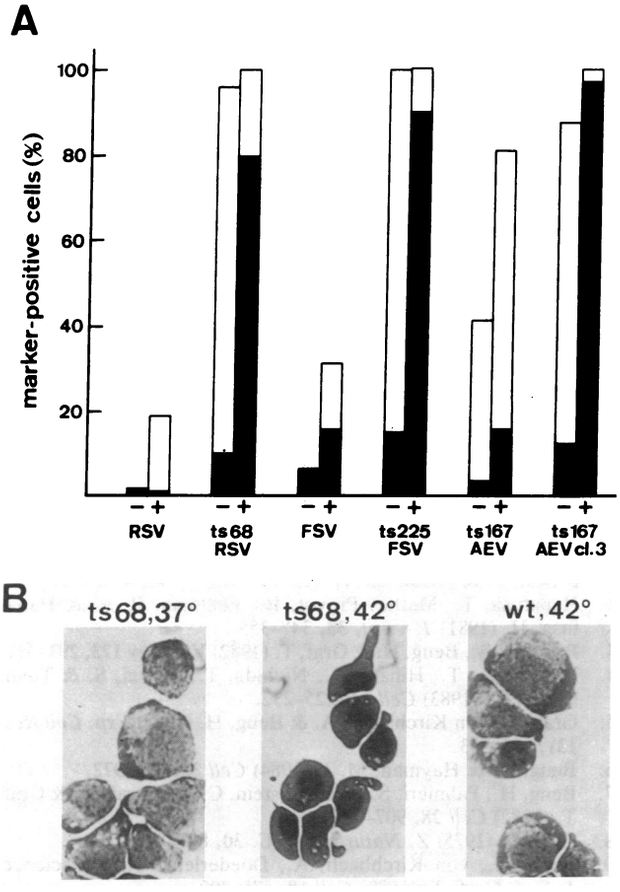

FIG. 4. Anemic serum-dependent terminal differentiation of virus-transformed erythroblasts shifted to nonpermissive temperature. ts or wt virus-infected cells were incubated at 42°C for 4 days in medium either containing (+) or lacking (−) anemic chicken serum. An aliquot of cells from each culture was then cytocentrifuged and stained as described in the legend to Fig. 3. (*A*) Open bars indicate proportion of erythroid cells containing hemoglobin; solid bars indicate proportion of terminally differentiated (erythrocyte-like) cells. (*B*) RSV-transformed erythroblasts at permissive and nonpermissive temperature, prepared as described in legend to Fig. 3 and photographed under green light.

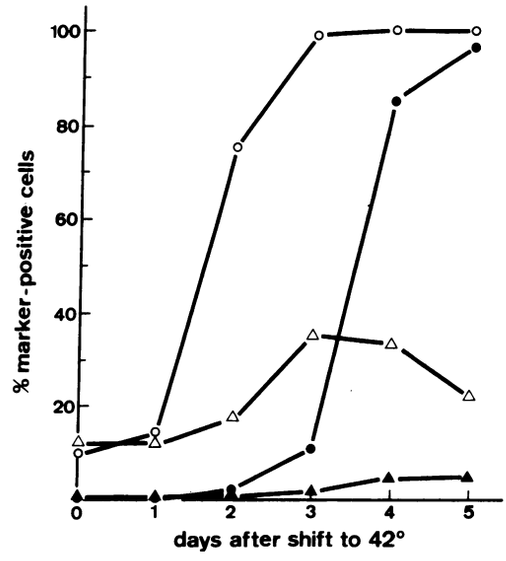

FIG. 5. Kinetics of hemoglobin induction in ASV-transformed erythroblasts shifted to nonpermissive temperature. tsRSV- or wtRSV-transformed erythroblasts were incubated at 42°C in CFU-E medium containing anemic chicken serum. At intervals of ≈24 hr, aliquots from each culture were cytocentrifuged and stained. ▲ and △, wtRSV cells; ● and ○, ts68 cells; open symbols, proportion of cells containing hemoglobin; closed symbols, proportion of erythrocyte-like cells.

erythroblasts preselected for its ability to differentiate well at 42°C (13) was included as a positive control. Four days later, aliquots from each culture were evaluated for the proportions of erythroblasts, reticulocytes, and erythrocytes according to their morphology and hemoglobin content (17).

The results of this experiment are illustrated in Fig. 4. Cultures transformed with either ts68RSV or ts225FSV and shifted to 42°C in the presence of anemic serum contained essentially 100% differentiated cells, 80%–90% of which were erythrocytes. In the absence of anemic serum, ≈80% of the cells reached the reticulocyte stage but relatively few (10% for tsRSV, 17% for tsFSV) were terminally differentiated. Similar results were obtained with the ts167AEV clone used as a control. None of the control cultures maintained at 37°C contained increased levels (>10%) of differentiated cells (data not shown). It should be noted that shifted ts cell populations generally showed extensive death, which was, however, much less pronounced in the presence of anemic serum. wtRSV-transformed populations showed essentially no increase in the proportions of reticulocytes or erythrocytes after shift to 42°C in the absence of anemic serum, while in its presence the proportion of both of these differentiated cell types were slightly elevated. wtFSV-transformed cells differentiated somewhat more at 42°C, particularly in the presence of anemic serum; the latter cultures contained ≈18% erythrocyte-like cells and ≈15% reticulocyte-like cells.

We also examined the time course of differentiation in mass cultures of wtRSV- or ts68RSV-transformed cells shifted to 42°C in the presence of anemic serum. The proportion of differentiated cells was monitored daily for 5 days by evaluating benzidine-stained cytocentrifuge preparations. The results of this experiment, which are shown in Fig. 5, indicate that ≈80% of the cells in the ts68RSV-transformed population were terminally differentiated by day 4 and that essentially all of the cells had differentiated into erythrocyte-like cells by day 5.

DISCUSSION

The data presented in this paper indicate that the ability to transform avian erythroid cells is not restricted to viruses carrying the *erbB* gene but is also exhibited by other oncogenes of the *src* family. It is perhaps surprising that the erythroid-transforming capacity of the ASVs was not seen until now. We attribute this to two factors. First, the improved conditions recently devised for the culture of avian erythroid cells support the growth of most ASV-transformed bone marrow cells, while the standard media tested so far do not. Second, our *in vivo* experiments were designed to minimize the extent of sarcoma formation at the site of inoculation, which presumably enabled the infected chickens to live long enough for virus-transformed cells to be detected in the peripheral blood.

Clonal populations transformed by RSV, FSV, PRCII, or AEV-H and cultured in the absence of anemic serum contain cells at early, intermediate, and late stages of differentiation, suggesting that the more mature cells in these cultures arise by spontaneous differentiation in an erythropoietin-independent fashion. These data also indicate that oncogenes of the *src* family transform erythroid cells without totally blocking their capacity to differentiate. In contrast, AEV-ES4-transformed bone marrow cells consist almost exclusively of immature erythroblasts, suggesting that the *erbA* gene may co-

operate with *erbB* in blocking erythroid cell differentiation, as also suggested earlier (3). The presence of *erbA* may also account for the observation that AEV-ES4-transformed erythroblasts have simpler growth requirements in culture than do ASV- and AEV-H-transformed erythroid cells, although the precise nature of these differences is not yet clear. We are presently investigating whether *erbA* can cooperate with oncogenes other than *erbB*.

Erythroblasts transformed with ts *src* or *fps* mutant viruses undergo terminal differentiation upon shift to the restrictive temperature, provided that an erythropoietin-like factor is present. It is noteworthy that nonclonal populations of tsRSV- or tsFSV-erythroblasts differentiated into 80%–100% erythrocytes after a shift to 42°C, while nonclonal populations of tsAEV-transformed cells generally contained no more than 20% erythrocytes (refs. 13 and 17; this paper). Erythroid cells transformed by tsASVs therefore appear to be more uniform in their ability to undergo temperature-induced terminal differentiation than do tsAEV-ES4 cells.

The results with the ts mutants also indicate that, as in fibroblasts (28), the continuous presence of the src or fps transforming protein is required for the maintenance of transformation in erythroblasts. Experiments with additional ts mutants should reveal whether tyrosine kinase (or a related phosphorylation activity) is essential for erythroid cell transformation by ASVs. No tyrosine kinase activity has yet been described for the erbB protein, but the recent finding that the erbB product is a truncated form of the epidermal growth factor receptor (29), which is believed to function as a tyrosine kinase (30), suggests that phosphorylation may be of general importance in the transformation of erythroid cells by oncogene-transducing retroviruses.

Several groups have recently shown that certain murine retroviruses enhance erythroid colony formation *in vitro*. The Abelson leukemia virus, which carries the *src*-related *abl* gene and transforms B-lymphoid cells and fibroblasts (31), induces erythroid colonies after infection of mouse fetal liver cells (32). Mouse erythroid colonies are also induced by the Harvey and Kirsten sarcoma viruses (33) and by the Friend virus complex (34), none of which contain *src*-related sequences. However, it is questionable whether these murine erythroid colonies can be truly regarded as virus-transformed because, in contrast to the *src*- and *fps*-transformed avian erythroid colonies, they cannot be expanded in liquid culture. This difference in self-renewal capacity between virus-induced murine and avian erythroid cells may either reflect variations among the oncogenes themselves or between the two species.

We would like to thank Drs. H. Hanafusa, J. Neil, and T. Patschinsky for providing us with the sarcoma viruses used in these studies. AEV-H was a generous gift from Dr. K. Toyoshima. We also thank Dr. M. Hayman for the anti-erbB antiserum and Dr. R. Kurth for the tumor-bearing rabbit serum. Carmen Walter and Claire Brady provided excellent technical assistance. P.K. was supported by a European Molecular Biology Organization fellowship, and B.A. was supported by Grant GR 746/1-1 from the Deutsche Forschungsgemeinschaft.

1. Graf, T. & Stehelin, D. (1982) *Biochem. Biophys. Acta* **651**, 245–271.
2. Teich, N., Wyke, J., Mak, T., Bernstein, A. & Hardy, W. (1982) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 785–998.
3. Frykberg, L., Palmieri, S., Beug, H., Graf, T., Hayman, M. J. & Vennstrom, B. (1983) *Cell* **32**, 227–238.
4. Sealy, L., Privalsky, M. L., Moscovici, G., Moscovici, C. & Bishop, J. M. (1983) *Virology* **130**, 155–178.
5. Yamamoto, T., Nishida, T., Miyajimi, N., Kawai, S., Ooi, T. & Toyoshima, K. (1983) *Cell* **35**, 71–78.
6. Shibuya, M. & Hanafusa, H. (1982) *Cell* **30**, 787–795.
7. Kitamura, N., Kitamura, A., Toyoshima, K., Hirayama, Y. & Yoshida, M. (1982) *Nature (London)* **297**, 205–208.
8. Anderson, S. M. & Scolnick, E. M. (1983) *J. Virol.* **46**, 594–605.
9. Radke, K., Beug, H., Kornfeld, S. & Graf, T. (1982) *Cell* **31**, 643–653.
10. Samarut, J. & Bouabdelli, M. (1980) *J. Cell Physiol.* **105**, 553–563.
11. Kawai, S. & Hanafusa, H. (1971) *Virology* **46**, 470–479.
12. Hanafusa, T., Mathey-Prevot, B., Feldman, R. A. & Hanafusa, H. (1981) *J. Virol.* **38**, 347–355.
13. Palmieri, S., Beug, H. & Graf, T. (1982) *Virology* **123**, 293–311.
14. Yamamoto, T., Hihara, T., Nichida, T., Kawai, S. & Toyoshima, K. (1983) *Cell* **34**, 225–232.
15. Graf, T., von Kirchbach, A. & Beug, H. (1981) *Exp. Cell Res.* **131**, 331–343.
16. Beug, H. & Hayman, M. J. (1984) *Cell* **36**, 963–972.
17. Beug, H., Palmieri, S., Freudenstein, C., Zentgraf, H. & Graf, T. (1982) *Cell* **28**, 907–919.
18. Graf, T. (1975) *Z. Naturforsch. C.* **30**, 847–849.
19. Beug, H., von Kirchbach, A., Doederlein, G., Conscience, J. F. & Graf, T. (1979) *Cell* **18**, 375–390.
20. Kornfeld, S., Beug, H., Doederlein, G. & Graf, T. (1983) *Exp. Cell Res.* **143**, 383–394.
21. Adkins, B. & Hunter, T. (1982) *Mol. Cell Biol.* **2**, 890–896.
22. Ellwart-Tschurtz, M. & Kurth, R. (1981) *J. Virol.* **39**, 950–953.
23. Beug, H., Kitchener, G., Doederlein, G., Graf, T. & Hayman, M. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6683–6686.
24. Laemmli, U. K. (1970) *Nature (London)* **227**, 777–785.
25. Fung, Y. K. T., Lewis, W. G., Kung, H. J. & Crittenden, L. B. (1983) *Cell* **33**, 357–368.
26. Hayman, M. J., Ramsey, G. M., Savin, K., Kitchener, G., Graf, T. & Beug, H. (1983) *Cell* **32**, 579–588.
27. Samarut, J. & Gazzolo, L. (1982) *Cell* **28**, 921–929.
28. Bishop, J. M. (1983) *Annu. Rev. Biochem.* **52**, 301–354.
29. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307**, 521–527.
30. Cohen, S., Fava, R. A. & Sawyer, S. T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6237–6241.
31. Rosenberg, N. (1982) *Curr. Top. Microbiol. Immunol.* **101**, 93–126.
32. Waneck, G. L. & Rosenberg, N. (1981) *Cell* **26**, 79–89.
33. Hankins, W. D. & Scolnick, E. M. (1981) *Cell* **26**, 91–97.
34. Hankins, W. D. & Troxler, D. (1980) *Cell* **22**, 693–699.