Pattern of Chick Gene Activation in Chick Erythrocyte Heterokaryons

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ABSTRACT The reactivation of chicken erythrocyte nuclei in chick-mammalian heterokaryons resulted in the activation of chick globin gene expression. However, the level of chick globin synthesis was dependent on the mammalian parental cell type. The level of globin synthesis was high in chick erythrocyte-rat L6 myoblast heterokaryons but was 10-fold lower in chick erythrocyte-mouse A9 cell heterokaryons. Heterokaryons between chick erythrocytes and a hybrid cell line between L6 and A9 expressed chick globin at a level similar to that of A9 heterokaryons. Erythrocyte nuclei reactivated in murine NA neuroblastoma, 3T3, BHK and NRK cells, or in chicken fibroblasts expressed <5% chick globin compared with the chick erythrocyte-L6 myoblast heterokaryons. The amount of globin expressed in heterokaryons correlated with globin mRNA levels. Hemin increased beta globin synthesis two- to threefold in chick erythrocyte-NA neuroblastoma heterokaryons; however, total globin synthesis was still <10% that of L6 heterokaryons.

Distinct from the variability in globin expression, chick erythrocyte heterokaryons synthesized chick constitutive polypeptides in similar amounts independent of the mammalian parental cell type. ~40 constitutive chick polypeptides were detected in heterokaryons after immunopurification and two-dimensional gel electrophoresis. The pattern of synthesis of these polypeptides was similar in heterokaryons formed by fusing chicken erythrocytes with rat L6 myoblasts, hamster BHK cells, or mouse neuroblastoma cells. Three polypeptides synthesized by non-erythroid chicken cells but less so by embryonic erythrocytes were conspicuous in heterokaryons. Two abundant erythrocyte polypeptides were insignificant in non-erythroid chicken cells and in heterokaryons.

The introduction by cell fusion of a dormant chick erythrocyte nucleus into a mammalian cell cytoplasm results in its reactivation. This process is characterized by increases in nuclear size, activation of RNA synthesis, formation of nucleoli and reexpression of chick genes (1–3). The activation is believed to be caused by an accumulation of mammalian nucleospecific proteins in the chick nucleus (4, 5). Erythrocyte reactivation in heterokaryons has been studied both early after fusion and for longer periods after inhibiting mammalian DNA replication by X-irradiation or mitomycin C pretreatment (1–7). Chick gene expression in heterokaryons has been described, including expression of α- and β-globins (7), surface antigens (8), hypoxanthine phosphoribosyl transferase (9), and rRNA (6). It has been unclear, however, whether the pattern of chick gene expression is variable after reactivation of erythrocyte nuclei in different mammalian cell cytoplasms.

In this study we have investigated the pattern of chick gene expression in various erythrocyte heterokaryons. The levels of chick globin synthesis in different heterokaryon cultures have been determined and related to the synthesis of nonglobin chick polypeptides. Chick polypeptides were immunopurified from heterokaryon lysates with various antisera and subjected to gel electrophoresis. By this protocol we have studied the synthesis of ~40 polypeptides in chick erythrocyte heterokaryons. Our findings demonstrate that chick globin gene expression is variable in heterokaryons formed by fusing chick erythrocytes with different mammalian cell lines. In contrast, the levels and distributions of nonglobin polypeptide synthesis were similar in different heterokaryon cultures.

MATERIALS AND METHODS

Cell Cultures

L6J1 is a subclone of Yaffe's L6 rat myoblast line (10) that was isolated in our laboratory (11). The tetraploid, nonmyogenic α-amanitin resistant L6 variants, 420 and 431 (12) were kindly provided by Dr. Mark Pearson. NA is a hypoxan-

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thine phosphoribosyl transferase-deficient subline of mouse C1300 neuroblastoma (13). BHK, 3T3, and NRK are established hamster, mouse, and rat lines, respectively. Primary cultures of chick fibroblasts were prepared from 10-d embryos and were used at low passage number. BB2 is a hybrid clone derived from a fusion between A9 and L6. This nonmyogenic line contains copies of all L6 chromosomes (14). All cells were grown in Dulbecco's modified Eagle's Medium, DME, supplemented with 10% fetal calf serum (FCS) and were mycoplasma-free as judged by Hoechst 33258 staining (15).

Cell Fusion

Cells were plated at a density of 5×10^4 cells per cm^2 and treated with mitomycin C at 0.2 μg/ml (L6, 420, 431, 3T3, NRK, BHK and BB2) or 1 μg/ml (NA and chick fibroblasts) for 16 h before fusion. Monolayers were then washed extensively with phosphate-buffered saline (PBS) and fused to erythrocytes from 16–18-d chick embryos. In some experiments it was important to remove contaminating cells from the erythrocyte preparations. Such cells will otherwise attach during the fusion procedure and grow. In some early experiments, the synthesis of chick polypeptides from contaminating chicken cells was detected in mock-fused control preparations. These contaminants could be removed by filtering chick erythrocyte preparations through lens paper and plating them in tissue culture dishes. Nonadhering cells were removed after overnight incubation and used for fusion experiments.

Cell fusion was carried out as described previously (5, 7) using UV-inactivated Sendai virus. Excess erythrocytes were removed after 16 h by washing with PBS, and both fused and parallel nonfused controls were maintained in DME plus 10% FCS. Fusion frequencies were determined from May Grünwald/Giemsa-stained cover slips and were generally between 50 and 100% (chick nuclei/mammalian nuclei). In some experiments, 0.1 mM hemin or 1% dimethyl sulfoxide, DMSO, was added to the cultures 24 h postfusion.

Immunoprecipitation and Gel Electrophoresis

Heterokaryons in 5-cm diameter dishes were labeled overnight with 20–40 μCi/ml of ^{35}S-methionine (Amersham International, Amersham, Buckinghamshire, England) at various times postfusion. Erythrocytes from 7–8-d chicken embryos were labeled with 1.5 mCi/ml of ^{35}S-methionine for 16 h. After labeling, cells were lysed and proteins were immunoprecipitated using protein A-Sepharose (Pharmacia, Uppsala, Sweden) as described (7). Globin immunoprecipitates were eluted from the protein A-Sepharose with 60 mM Tris-HCl, pH 6.8, 2.3% sodium dodecyl sulphate (SDS), 5% vol/vol 2-mercaptoethanol and 10% vol/vol glycerol and separated on 15% SDS-polyacrylamide slab gels. For two-dimensional gel analysis of immunoprecipitates, the polypeptides were eluted with lysis buffer (16) and separated, in the first dimension, on isoelectric focusing gels prepared using a 4:1 mixture of 5–7 and 3.5–10 Ampholines (LKB, Bromma, Sweden) and, in the second dimension, on 11.5% SDS-polyacrylamide gels (16). All gels were fluorographed with EN^{3}HANCE (New England Nuclear, Boston, MA) and exposed to Fuji X-ray film at −70° C. Globin levels were quantitated by scanning fluorograms with a Joyce-Loebl densitometer. Quantification and normalization of globin levels between the different heterokaryons was performed by dividing the maximal peak height obtained from densitometry with the fusion frequency times the total trichloroacetic acid-insoluble radioactivity subjected to immunoprecipitation.

Partial proteolytic digestion of polypeptides in gel slices was carried out as described by Cleveland et al. (17). 0.24 μg of papain (Sigma Chemical Co., St. Louis, MO) was used for each digestion.

Antisera

All antisera used were raised in rabbits. Antiserum AC-1 was obtained following four weekly subcutaneous immunizations of a rabbit with primary chicken embryo cells (mostly fibroblasts and myoblasts) in Freund's adjuvant and one intravenous boost without adjuvant. Antisera directed against chick hemoglobin, chick ovalbumin, and chick serum were purchased from Cappel Laboratories Inc. (Cochranville, PA). The histone H5 serum was kindly provided by Dr. Thomas Graf (German Cancer Research Center, Heidelberg, W. Germany).

RNA Blot Hybridization

RNA was extracted from heterokaryon cultures with phenol at 65°C (18) and subsequently denatured, electrophoresed, and blotted to nitrocellulose filters (19, 20). Filters were hybridized with ^{32}P-labeled nick-translated (10^8 cpm/μg) chicken α^A-globin probes for 18 h at 42°C in 50% formamide and 10% dextran sulfate (21). Filters were washed, dried, and autoradiographed at −70°C using Fuji X-ray film and Du Pont Lightning Plus intensifying screens. Plasmid α-14, an adult α^A-globin chick genomic insert in pBR322 (22), was provided by Dr. Harold Weintraub (Fred Hutchison Cancer Research Center). Recombinant DNA was handled in accordance with the Swedish guidelines for recombinant DNA research.

RESULTS

Detection of Chick Gene Expression

The primary objective of the present investigation was to study chick gene expression in chick erythrocyte heterokaryons. This was achieved by immunoprecipitating radiolabeled heterokaryon lysates using various antisera directed against chicken polypeptides followed by polyacrylamide gel electrophoresis (PAGE) and fluorography. To allow for chick erythrocyte reactivation, mammalian parental cells were treated with mitomycin C before fusion. This treatment prevents overgrowth of nonfused mammalian cells and makes it possible to maintain heterokaryons in culture for more than 3 wk (7).

Fig. 1 demonstrates the detection of chick polypeptide synthesis in heterokaryons between mitomycin C-treated BHK hamster cells and chicken erythrocytes. Heterokaryon cultures were labeled with ^{35}S-methionine at 10 d postfusion; lysates were immunopurified with an antiserum prepared against chicken embryo cells (serum AC-1) and subjected to two-dimensional gel electrophoresis. Fig. 1*a* represents a hetero-

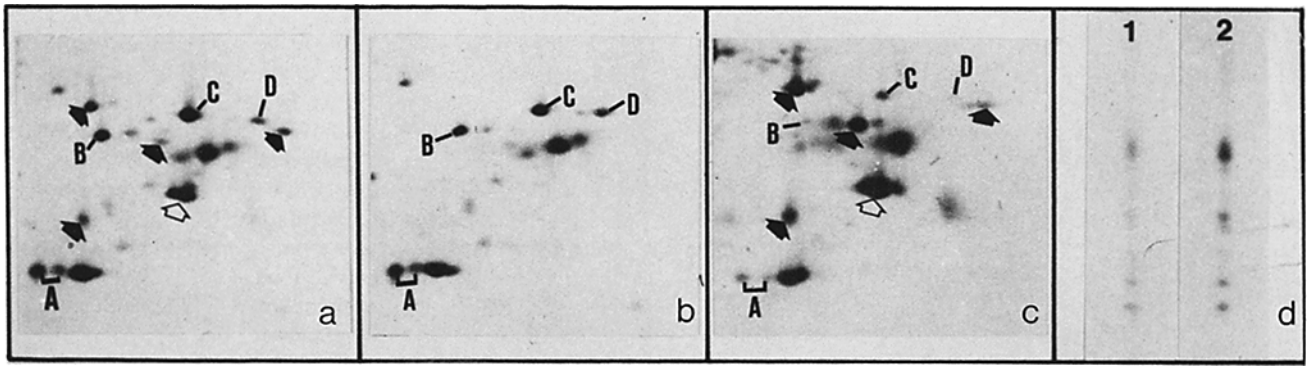

FIGURE 1 Chicken polypeptide synthesis in chicken erythrocyte-BHK heterokaryons. (*a*) Chicken erythrocyte-BHK cell heterokaryons labeled 10 d after fusion. (*b*) BHK mock-fused control (treated identically as in *a*, except that the Sendai virus was omitted). (*c*) Mix between primary chicken embryo cells and BHK cells. All samples were immunopurified with serum AC-1. Arrows indicate chicken polypeptides; *a–d* indicate some background spots of BHK origin. Only a minor part of each fluorogram is shown (the extreme right chicken polypeptide corresponds to spot *26* in Fig. 6 *a*). (*d*) Limited papain digestion of heterokaryon and chicken cell polypeptides. The spots marked by open arrows in *a* and *c* were digested in situ and the fragments were separated on a 15% SDS-polyacrylamide gel. Lane *1*: the heterokaryon polypeptide; lane *2*: the chicken embryo cell polypeptide.

karyon polypeptide separation, and Fig. 1 *b* is the corresponding mock-fused control (treated identically except for the omission of the fusing agent). The spots marked by arrows in Fig. 1 *a* are not present in the control gel, indicating that they are dependent on heterokaryon formation. Some background spots (*A–D*) are present in both gels. In order to examine whether the heterokaryon specific spots may represent chick polypeptides, a mixture of primary chicken embryo cell and BHK cell lysates was immunopurified with serum AC-1 (Fig. 1 *c*). This analysis shows that polypeptides comigrating with the heterokaryon specific polypeptides in fact are present in chicken cells.

As a further identification, the putative chicken polypeptide marked by an open arrow in Fig. 1 *a* was subjected to partial proteolytic digestion (Fig. 1 *d*). Lane *1* is a limited papain digestion of this heterokaryon polypeptide, and lane *2* a digestion of the corresponding chicken embryo cell polypeptide. It is evident from this analysis that the heterokaryon specific polypeptide is indeed a chicken gene product. The anti-chicken cell serum AC-1 allows the detection of constitutive proteins in heterokaryons (see below) and provides a tool for determining the degree of reactivation in chick erythrocyte heterokaryons.

Expression of Chick Globin

We have previously observed that reactivation of chicken erythrocyte nuclei in heterokaryons results in globin gene expression (7). In the present experiments we wished to establish whether the extent of globin gene expression varies depending on the mammalian cytoplasm where the erythrocyte nucleus is reactivated. For this purpose, heterokaryon cultures were assayed for both the levels of globin and constitutive protein synthesis (Fig. 2). This was accomplished by immunoprecipitating the same radiolabeled heterokaryon extract with an anti-chick hemoglobin serum (Fig. 2 *a*) and with the anti-chicken cell serum (Fig. 2 *b* and *c*). From this analysis it was evident that significant synthesis of chicken α- and β-globin occurred at 10 d postfusion in chick erythrocyte-L6 myoblast heterokaryons (Fig. 2 *a*, lane *2*) whereas globin synthesis in chick erythrocyte-NA neuroblastoma heterokaryons was barely detectable (Fig. 2 *a*, lane *1*). This variability could not be explained by differences in the degree of erythrocyte reactivation because comparable levels of chick polypeptides precipitable with the anti-chicken cell serum were synthesized by both types of heterokaryons (Fig. 2 *b* and *c*). These polypeptides (arrowheads) were found in heterokaryons (Fig. 2 *b* and *c*), but not in control mock-fused cultures (Fig. 2 *d* and *e*). Therefore, the variability in globin expression is in contrast to the similar levels of other chick polypeptides.

The levels of globin synthesis in different chicken erythrocyte heterokaryons were quantitated by densitometry (Table I). Heterokaryons involving L6 or two different α-amanitin-resistant variants of L6 (420 and 431) showed the highest levels of chick globin synthesis. Heterokaryons between mouse A9

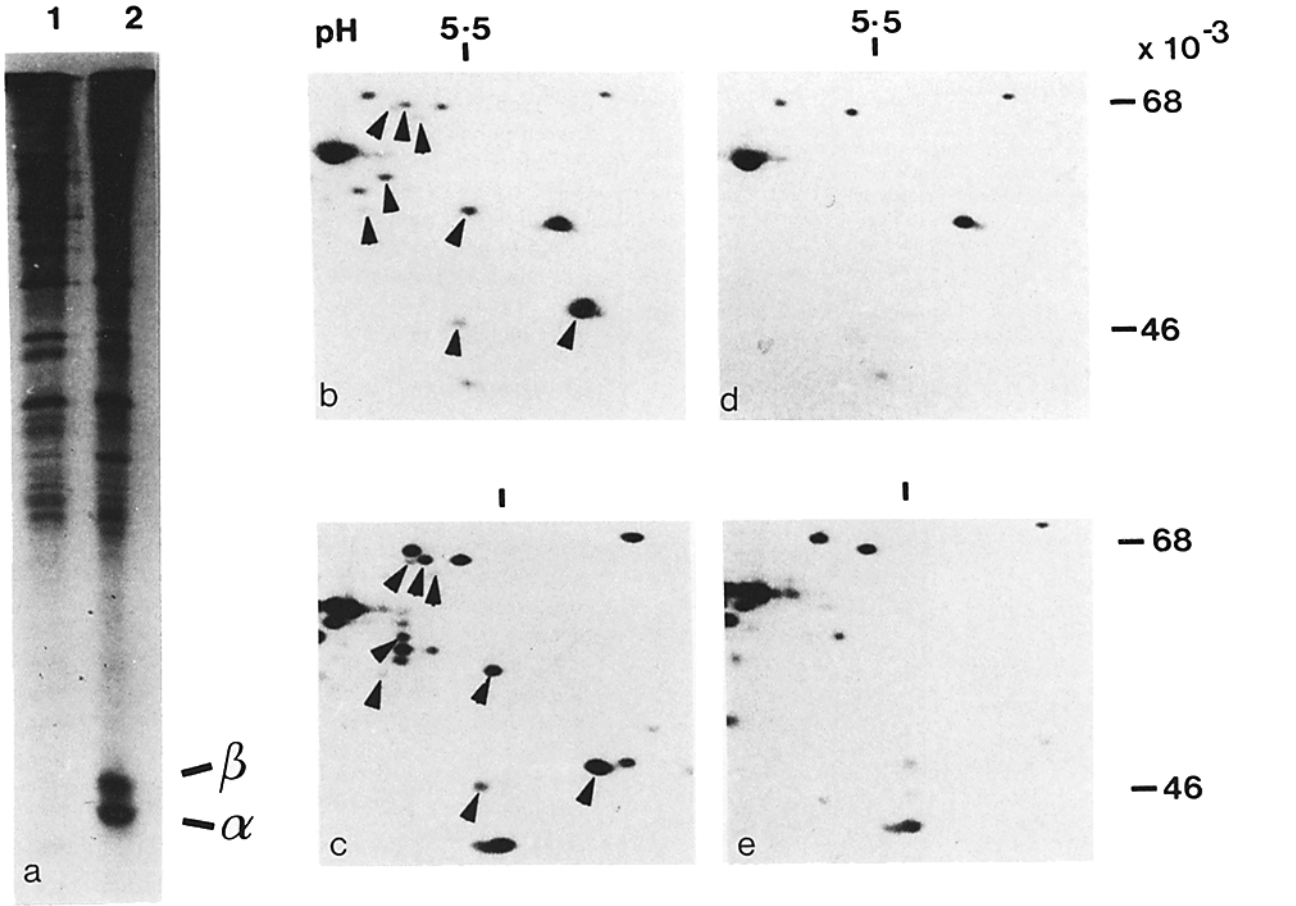

FIGURE 2 Heterokaryon cultures were labeled with ^{35}S-methionine at 10 d postfusion and lysates were immunoprecipitated and separated by gel electrophoresis. Heterokaryon lysates were divided and assayed both for chick globin (*a*) and by two-dimensional gel electrophoresis (*b*). (*a*) chick globin immunoprecipitates; lane *1*: chick erythrocyte-NA heterokaryons; lane *2*: chick erythrocyte-L6J1 heterokaryons. (*b–e*) anti-chicken cell immunoprecipitates from (*b*) chick erythrocyte-NA heterokaryons; (*c*) chick erythrocyte-L6J1 heterokaryons; (*d*) NA nonfused control; (*e*) L6J1 nonfused control. Chicken polypeptides present in heterokaryons but not in control cultures are indicated in *b* and *c* with arrowheads.

cells and chicken erythrocytes had ~15% the globin synthesis of L6 heterokaryons. Chick erythrocytes fused to BB2, a hybrid between A9 and L6 (14), had globin levels similar to those of A9 heterokaryons. This suggests that low globin expression is dominant over high, in heterokaryons. The levels of globin expression in heterokaryons involving NA, BHK, NRK, 3T3, and primary chick fibroblasts were <5% those of chick erythrocyte-L6 heterokaryons. The levels of globin synthesis were related to total chicken polypeptide synthesis in heterokaryons by comparing the radioactivity incorporated into globin with that incorporated into polypeptides precipitated by the anti-chick cell serum (~10% of the polypeptides in chick fibroblasts were immunoprecipitated by this serum). Globin synthesis was estimated to be ~20–50% and 1–3% of the total chick polypeptide synthesis in L6 and NA heterokaryons, respectively.

TABLE I

Globin Expression Chick Erythrocyte Heterokaryons

	Parental cell	Globin expression
L6J1	rat myoblast, diploid, myogenic	100
420, 431	rat myoblast, tetraploid, nonmyogenic	100
A9	mouse L-cell	15
BB2	L6J1 × A9 hybrid (14)	10
NA	mouse neuroblastoma	5
BHK	hamster	5
3T3	mouse	5
NRK	rat kidney	5
Chicken primary fibroblasts		5

Globin expression is relative to L6 heterokaryons, which is defined as 100%. Globin was quantified by measuring the maximal peak intensity of alpha globin using a Joyce-Loebl densitometer and normalized by dividing by the fusion frequency times the total cpm subjected to immunoprecipitation. The levels indicated for the low globin producers are maximum values. In many experiments these values were two- to threefold lower.

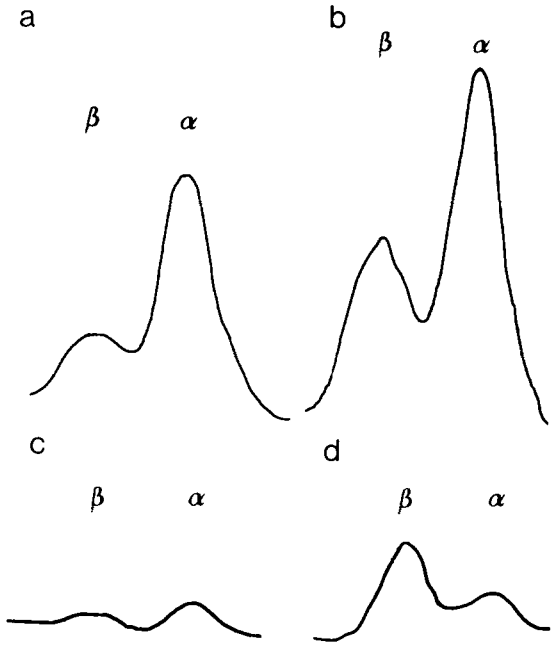

FIGURE 3 Globin immunoprecipitates from chick erythrocyte-L6J1 (*a* and *b*) or chick erythrocyte-NA (*c* and *d*) heterokaryons that had been cultured in the presence (*b* and *d*) or absence (*a* and *c*) of 0.1 mM hemin were separated by gel electrophoresis and the resulting fluorograms were scanned by a Joyce-Loebl densitometer. The gels were scanned using two different sensitivities on the densitometer: the levels of globin expression can therefore not be compared between L6J1 and NA heterokaryons.

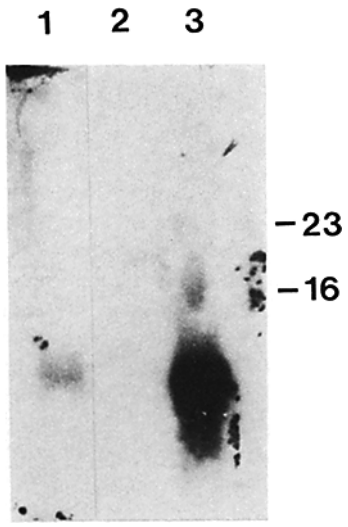

FIGURE 4 Total RNA was isolated from chick erythrocyte heterokaryons 11 d postfusion (lane *1* and *2*) or from 10-d embryo erythrocytes (lane *3*) and separated by agarose gel electrophoresis. RNA was transferred to a nitrocellulose filter and hybridized with an α^A-globin probe. Lane *1*: chick erythrocyte-L6J1 heterokaryons (25 μg RNA loaded, fusion frequency: 36%); lane *2*: chick erythrocyte-A9 heterokaryons (12 μg RNA loaded, fusion frequency: 77%). Composite photograph of the same X-ray film; exposure time: 8 d.

The effect of the globin inducers hemin and DMSO on heterokaryons was determined. The densitometric scans (Fig. 3) indicate that hemin was capable of stimulating α and β-globin synthesis in L6J1 and NA heterokaryons. The effect was most pronounced on β-globin synthesis. DMSO alone had no significant effect on globin synthesis (data not shown). Despite the two- to three fold increase in β-globin synthesis by hemin, total globin synthesis in hemin-treated chick erythrocyte-NA heterokaryons was still <10% that of the chick erythrocyte-L6 heterokaryons. Therefore, the low levels of globin synthesis seen in chick erythrocyte-NA heterokaryons were not reversible with globin inducers.

Globin mRNA Levels

RNA was extracted from heterokaryons, and α^A-globin mRNA was detected by blot hybridization (Fig. 4). The amount of RNA subjected to analysis was corrected for the fusion frequency so that RNA from the same chick nuclear equivalents was analyzed. Globin mRNA was detected in chick erythrocyte-L6 heterokaryons (lane *1*), but not in chick erythrocyte-A9 heterokaryons (lane *2*). Therefore, the levels of chick globin synthesis in heterokaryons appeared to reflect the levels of globin mRNA. Although we cannot exclude a more rapid rate of globin mRNA degradation in the low globin producers, our findings suggest that regulation of globin expression occurs at the level of transcription.

Chick Polypeptides Precipitated by the AC-1 Serum

The tissue specificity of the polypeptides precipitated with the AC-1 anti-chicken cell serum was studied by labeling primary cultures of chicken cells (mostly fibroblasts and myoblasts) and chicken erythrocytes from 7–8-d old embryos (that still have a low level of protein synthesis) with ^{35}S-methionine. After immunopurification and two-dimensional gel analysis, ~30 major spots appeared on the fluorograms (Fig. 5). Almost

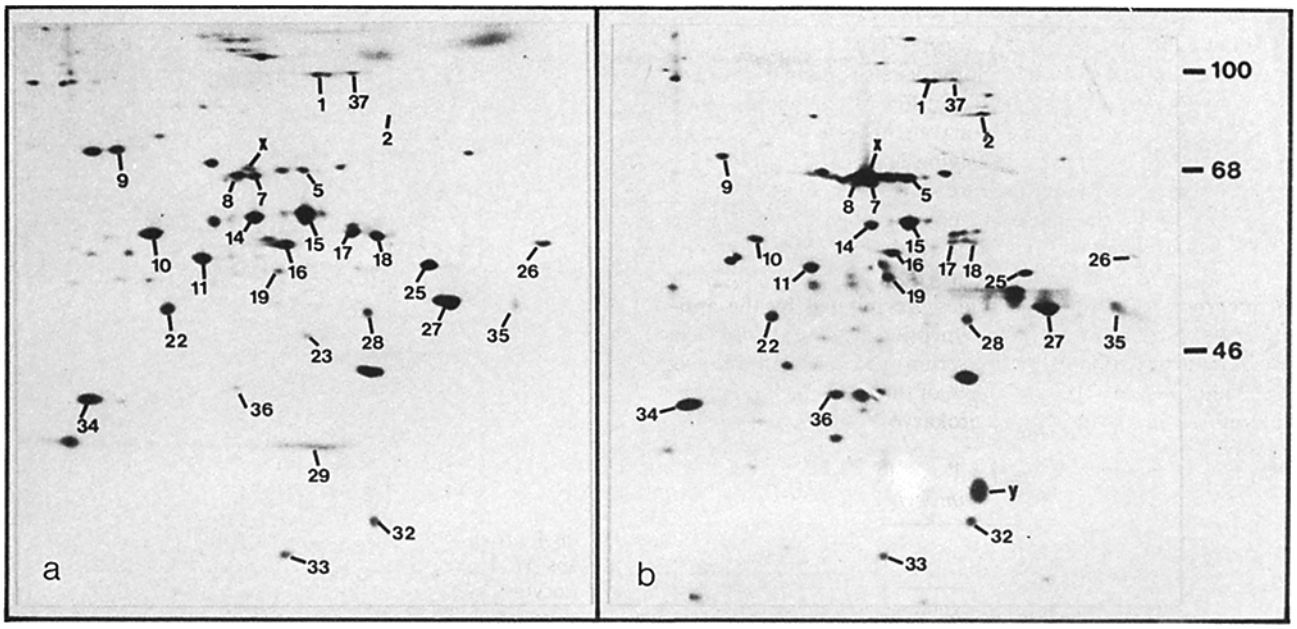

FIGURE 5 Immunopurification of ^{35}S-methionine-labeled proteins of (a) primary chicken embryo cells and (b) erythrocytes from 7-8-d-old chicken embryos with serum AC-1.

all polypeptides were synthesized by both cell populations. However, a number of differences in spot intensities were apparent between the primary chicken cell (Fig. 5 *a*) and the erythrocyte gels (Fig. 5 *b*): (*a*) spots *17* and *18* are conspicuous in the primary chicken cell gel but not in the erythrocyte gel; (*b*) spot *29* is stronger in primary chicken cells than in erythrocytes; (*c*) two dominating spots in the erythrocyte gels (*x* and *y*) are insignificant (although both were present in long exposures) in primary chicken cells.

Pattern of Chicken Gene Expression in Different Heterokaryons

The pattern of chick polypeptide synthesis was studied in heterokaryons between chicken erythrocytes and BHK hamster cells, rat L6J1 myoblasts or mouse NA neuroblastoma cells by immunoprecipitation with the AC-1 serum and two-dimensional gel electrophoresis. Fig. 6 *a* shows a polypeptide analysis of BHK heterokaryons 11 d after fusion. 36 spots are present in the heterokaryon gel, but absent in the mock-fused control (Fig. 6 *b*). Similar results were obtained in heterokaryons with rat L6J1 myoblasts (Fig. 6 *c*) and with mouse NA neuroblastoma cells (Fig. 6 *d*). The same heterokaryon-specific spots were present on these different fluorograms with some exceptions: (*a*) spots *30* and *31* were not present in NA heterokaryons (two components (*30'* and *31'*) with similar migration properties but with slightly lower molecular weights were present instead); (*b*) spot *23* was absent in L6J1 heterokaryons (a polypeptide with a slightly lower molecular weight was present instead [*23'*]); (*c*) spots *24* and *36* were not detected in L6J1 heterokaryons; (*d*) NA heterokaryons have a 50-kdalton polypeptide (spot *38*) not present in the other heterokaryons; (*e*) it could not be determined whether spot *1* was present in L6J1 and NA heterokaryons or whether spot *37* was present in BHK heterokaryons as background material veiled these positions. These experiments were repeated three to five times. The reproducibility between different gels was excellent.

The conclusions from these studies is that basically the same gene products (of the limited number that we were able to study) appeared to be expressed in all three types of heterokaryons. In addition to the differences in spot patterns described above, some differences in spot intensities occurred between the different gels. As an illustration of this, spots *9* and *25* appear to be equally strong in BHK heterokaryons (and also in L6J1), whereas spot *25* is very weak compared to spot *9* in NA heterokaryons. Furthermore, the pattern of polypeptide synthesis in heterokaryons appears to correspond to that found in the chick embryo cells, but less so to those found in chick erythrocytes (compare Figs. 5 and 6). Thus, spots *17* and *18* are conspicuous in chick embryo cells and in heterokaryons, but weak in chick erythrocytes. Spot *29* is present in chick embryo cells and heterokaryons, but is much weaker in erythrocytes. The dominating red cell spots *x* and *y* are weak in chick embryo cells and not detectable in heterokaryons.

Kinetics of Activation

To follow the time course of the synthesis of chicken polypeptides, chicken erythrocyte-rat L6J1 myoblast heterokaryons were labeled with ^{35}S-methionine at different times postfusion and immunopurified with the anti-chicken serum. Some chick spots on the two-dimensional gels were scanned with a densitometer. The result of this analysis is presented in Fig. 7. The synthesis of four quantified chick polypeptides increased several-fold between two and eight d postfusion. Visual inspection indicated that the other chick polypeptides followed the same kinetics of activation.

Immunopurification with Other Antisera

Using the AC-1 serum for immunoprecipitation, we were only able to study the synthesis of ~35 polypeptides in heterokaryons. It was necessary, however, to use this approach in order to detect the synthesis of chick polypeptides against the background of mammalian polypeptides. In an attempt to extend the range of chick polypeptides amenable for study, we prepared two additional rabbit anti-chicken sera. Both these antisera reacted against approximately the same spectrum of polypeptides as AC-1 although the synthesis of some additional polypeptides was detected in heterokaryons (not shown). None

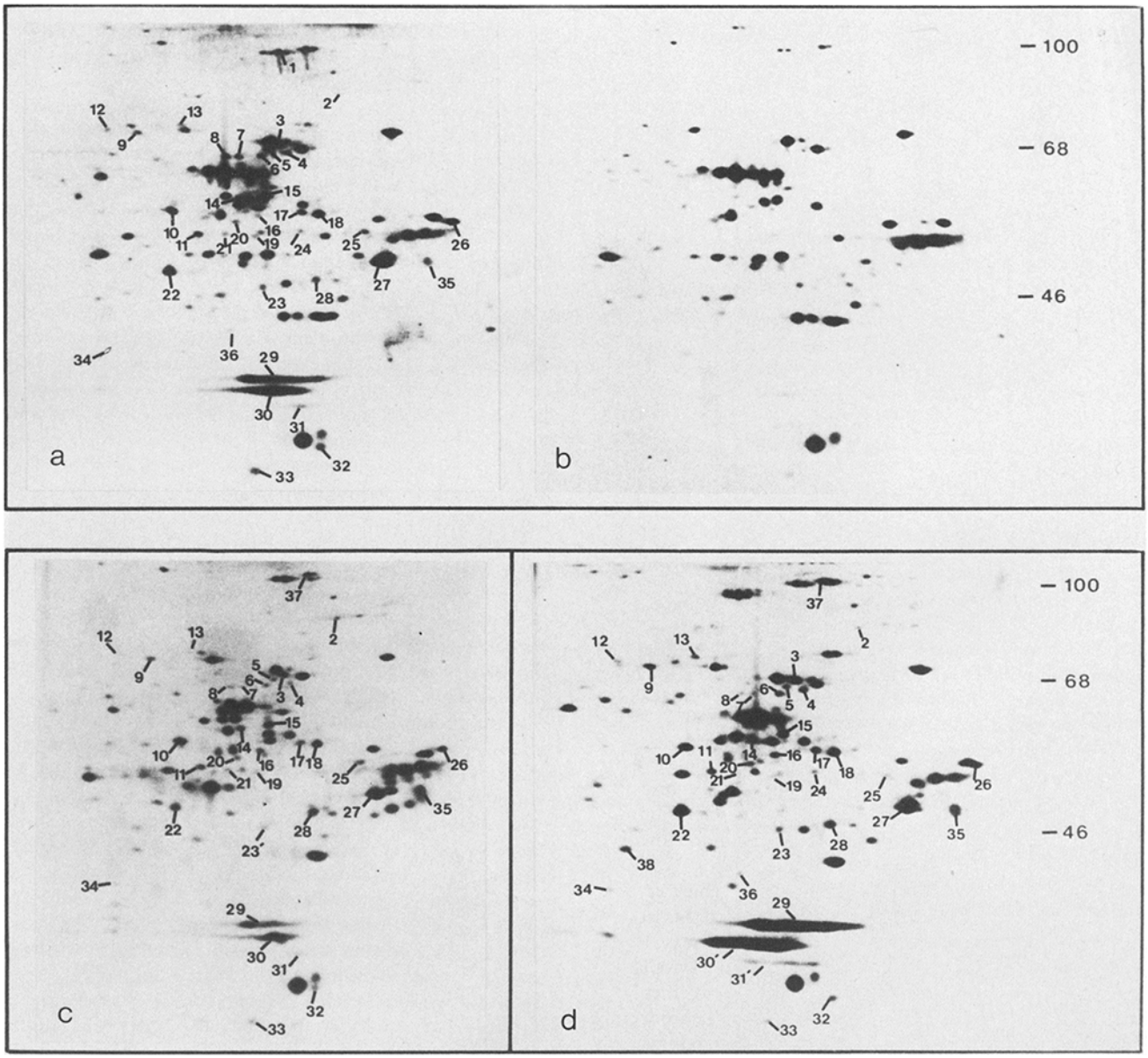

FIGURE 6 Immunopurification of ^{35}S-methionine-labeled proteins of heterokaryon lysates with serum AC-1. (*a*) Chicken erythrocyte-BHK heterokaryons; (*b*) BHK mock-fused control; (*c*) chicken erythrocyte-L6J1 rat myoblast heterokaryons; (*d*) chicken erythrocyte-NA mouse neuroblastoma heterokaryons. All cells were labeled at 11 d postfusion. Erythrocytes were obtained from 18-d-old chicken embryos.

of these additional polypeptides were specific for either erythroid or non-erythroid cells.

We have used the immunoprecipitation/two-dimensional-gel protocol in order to detect expression of other chicken polypeptides. Strongly labeled heterokaryon and mock-fused control lysates were immunoprecipitated with anti-chicken H5 serum, anti-chicken ovalbumin serum, and with an anti-serum directed against chicken serum proteins (Table II). We were unable to detect synthesis of any chicken polypeptides in heterokaryons using these antisera. Table II summarizes our phenotypic characterization of erythrocyte heterokaryons.

DISCUSSION

The findings in this and in our earlier study (7) demonstrate that the reactivation of chicken erythrocyte nuclei in mammalian cell cytoplasms results in the reexpression of chick globin genes. However, the level of globin expression varied depending on the mammalian parental cell type. L6 rat myoblast lines supported the highest amounts of chick globin synthesis. We estimated that globin synthesis represented between 20 and 50% of total chick polypeptide synthesis in these heterokaryons. Only 5–15% of the chick erythrocyte-L6 globin levels were expressed in the other heterokaryons. The variability in globin synthesis was reflected in the levels of α^A-globin mRNA.

Although the basis for regulation of globin expression remains unclear, it is apparent that this variability is in contrast to the constant levels of other chick polypeptides synthesized in the different heterokaryons. Cell hybrid studies have suggested that cytoplasmic regulators exist that are capable of inhibiting expression of facultative markers (for a review see reference 3). For example, Weiss and co-workers have reported extinction of rat albumin synthesis in cybrids after fusion of rat hepatoma cells with enucleated mouse fibroblasts (23) and

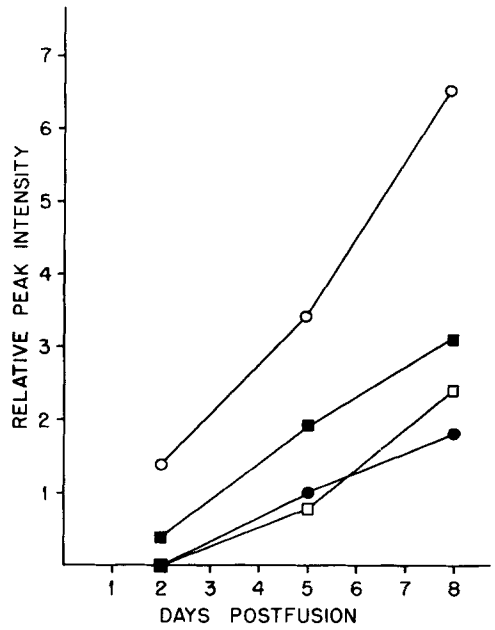

FIGURE 7 Increase in chicken polypeptide synthesis during reactivation. Chicken erythrocyte-L6J1 heterokaryons were labeled at varying times postfusion, and the proteins were immunopurified and subjected to two-dimensional gel electrophoresis. Some heterokaryon specific spots were scanned on the resulting fluorograms with a Joyce-Loebl densitometer. The intensity of these spots was plotted after correction for the amount of radioactivity that was submitted to immunoprecipitation and for exposure time.

in heterokaryons between rat hepatoma cells and mouse fibroblasts (24). Inhibition of albumin synthesis in the cybrids was short-lived and the synthesis was restored 48 h postfusion. The presence of cytoplasmic regulators could explain the low level of globin synthesis observed in most of the heterokaryons and would be consistent with our findings with the chick erythrocyte-BB2 (A9 × L6 hybrid) heterokaryons. That L6 is more permissive for expression of chick globin genes may be explained by the absence of such specific negative cytoplasmic regulators. We have attempted to investigate whether high levels of globin synthesis are observed following introduction of chick erythrocyte nuclei into other myoblast populations. These experiments were unsuccessful with chick and rat primary myoblasts due to fibroblast contaminations and unsatisfactory fusion and survival. The L8 rat myoblast line supported chick erythrocyte reactivation, however. These heterokaryons showed a level of globin synthesis similar to that of NA cells. It is therefore not clear whether the high levels of globin synthesis in chick erythrocyte-L6 heterokaryons are related to the myoblast phenotype. The high level of chick globin synthesis in heterokaryons with L6 was not a consequence of (*a*) L6 being myogenic since the α-amanitin-resistant L6 variants are nonmyogenic; (*b*) gene dosage: diploid and tetraploid L6 lines supported high levels of globin synthesis, whereas diploid chick fibroblasts and subtetraploid A9 and NA did not; (*c*) L6 synthesizing rat globin: neither L6 parental cells nor chick erythrocyte-L6 heterokaryons synthesized rat globin (data not shown).

The inhibition of globin expression in NA or A9 heterokaryons was not readily reversible by hemin or DMSO although some increases in globin synthesis were detected. Hemin has been demonstrated to increase globin synthesis in Friend cells, the human erythroleukemic line K562, and in hybrids between Friend × lymphoma cells (25–29). It is of interest that the hemin-related stimulation in our system was more significant for β-globin than for α-globin synthesis. Hemin has been demonstrated to selectively increase the transcription of the β-minor over the β-major globin genes in Friend erythroleukemia cells (27). Although it remains to be determined at what level hemin is acting in our system, it is clear that the low level of globin expression in chick erythrocyte-NA heterokaryons is not readily reversed by either hemin or DMSO.

The expression of globin genes has been studied intensively in somatic cell hybrids and the results have been difficult to interpret because both extinction and activation of globin gene expression can be due not only to regulatory phenomena, but also to chromosomal loss and in vitro selection. In the present studies, where these problems can be excluded, it can still be demonstrated that globin expression is variable and dependent on the mammalian parental cell type. This finding is in contrast to the constant levels of expression of other chick polypeptides in the heterokaryons and demonstrates the independent regulation of the chick globin genes. The basis for this regulation and the identification of the regulatory factors involved remain to be determined.

The pattern of synthesis of some 35 chick nonglobin polypeptides was studied by immunoprecipitation with anti-chicken cell antibodies. The AC-1 serum precipitated ~30 major polypeptides in chicken cells. Some of these polypeptides were expressed at different levels in chicken erythrocytes and chicken embryo cells. We believe that these differences are due to varying levels of constitutive gene expression in the two cell populations and are unlikely to represent the type of tissue specific gene expression that can be exemplified by globin or ovalbumin. The two-dimensional gel analysis of chicken polypeptide synthesis showed that largely the same chicken polypeptides were synthesized in three different types of heterokaryons. The pattern of chicken polypeptide synthesis in heterokaryons was similar to the pattern of non-erythroid chicken cell expression, but was different from that of erythrocytes. It would then appear that the level of expression of constitutive gene products is altered after reactivation to resemble the expression normally seen in non-erythroid cells. These changes did not appear to be related to the expression of chicken globin as both the heterokaryons that supported high levels of globin expression (L6J1) and those that did not (BHK and NA) showed largely the same pattern of chick polypeptide synthesis.

Some differences in spot intensities were apparent between

TABLE II

Synthesis of Chick Polypeptides in Erythrocyte Heterokaryons

Chick polypeptide	Heterokaryons with L6J1	Heterokaryons with NA	Reference cells: Chick embryo cells	Reference cells: Erythrocytes
Globin (references 5 and 6)	+	+/−	−	+
Histone H5	−	−	−	+
Spots *17, 18, 29*	+	+	+	+/−
Spots *x, y*	−	−	+/−	+
Ovalbumin*	−	N.D.	N.D.	N.D.
Chicken serum proteins	−	N.D.	N.D.	N.D.

(+): High level of synthesis; (+/−): low level of synthesis; (−) not detected. N.D.: not done.

* The anti-ovalbumin serum precipitates both in vitro translated ovalbumin and commercially obtained ^{14}C-methylated ovalbumin.

gels from different heterokaryons. This would suggest that the level of chicken polypeptide synthesis in heterokaryons is to some extent influenced by the mammalian cell type. However, these differences might also be due to posttranscriptional events. It is evident that more chick spots are observed in heterokaryons than in primary chicken cells. Almost all heterokaryon specific spots are present in more than one type of heterokaryon (for example, spots *6*, *20*, *30*, and *31*). Because the mammalian parental cells were from three different species, it appears unlikely that these polypeptides are specified by the mammalian nucleus. One possibility is that posttranscriptional events occur in the heterokaryons that lead to the synthesis of immunologically related polypeptides with slightly different migration behavior on two-dimensional gels. For example, heterokaryon spots *30* and *31* (and in addition *30'* and *31'*) cannot be detected in chicken cells even after very long exposure of the fluorograms. These spots have similar pIs and molecular weights as spot *29* (and they all appear to be rather insoluble in high concentrations of urea as indicated by the smearing in the first dimension). Therefore spots *30* and *31* may represent the same gene product as spot *29* which, although it is slightly altered, still reacts with the AC-1 serum. We have found that polypeptide *29*, contrary to *31*, is phosphorylated (unpublished observation). Phosphorylation alone, however, could not explain the difference in molecular weights between these polypeptides.

Our findings suggest that, although the globin genes are activated in the chick nucleus, the pattern of constitutive chick gene expression resembles that of non-erythroid cells. We were unable to detect any synthesis of erythrospecific histone H5 in heterokaryons. We were also unable to find synthesis of chicken ovalbumin or serum albumin, suggesting that the activation process is specific and does not lead to expression of all chicken genes. However, it must be pointed out that the level of overall chick protein synthesis in the heterokaryons is quite low. It is therefore possible that these polypeptides are synthesized at a low level.

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