

# Migration of Rat RNA Polymerase I into Chick Erythrocyte Nuclei Undergoing Reactivation in Chick-Rat Heterokaryons

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**ABSTRACT** Transcriptionally inactive chick erythrocyte nuclei were reactivated by Sendai virus-induced fusion of erythrocytes with rat L6J1 myoblasts. We used antibodies to trace the appearance of a specific protein engaged in transcription of a defined class of genes, those coding for rRNA, during reactivation. Using immunofluorescence microscopy, we found increasing amounts of rat RNA polymerase I to appear, during a certain period of time after fusion, in the reforming nucleoli of the chick nuclei. Amounts of rat RNA polymerase I sufficient to be detected by immunofluorescence microscopy had accumulated in the newly developed chick nucleoli 72–190 h after fusion was initiated. This time interval coincides with the time when chick rRNA synthesis can first be detected. The results raise the possibility that during these stages of the reactivation process chick rRNA genes are transcribed by heterologous RNA polymerase I molecules of rat origin.

Mature chick erythrocytes are terminally differentiated cells in which the nuclear chromatin is tightly condensed, and in which replication, as well as transcription, have almost completely stopped (1). After fusion with actively transcribing mammalian cells, the nuclei of chick erythrocytes undergo a reactivation process characterized by an increase in nuclear size and protein content and by dispersion of chromatin and development of nucleoli (2–6). This increase of nuclear size is paralleled by a selective uptake of mammalian nucleus-specific proteins (4, 7) as well as by disappearance of some specific components of the chick erythrocyte chromatin (7, 8).

In the present study we examined the nucleocytoplasmic exchange of specific proteins during the reactivation of chick erythrocyte nuclei in heterokaryons. To this end we used an antiserum against rat RNA polymerase I (9), which does not cross-react with the corresponding enzyme from nonmammalian species (Fig. 1 *b*).<sup>1</sup> We show that rat RNA polymerase I molecules entered the chick nucleus during the reactivation process and accumulated in the reforming nucleolus.

## MATERIALS AND METHODS

**Cells:** L6J1, a myogenic subclone of Yaffe's L6 rat myoblast line (10), isolated in our laboratory by John Coleman (see reference 11), was cultured on Dulbecco's minimal essential medium (DME) containing 10% fetal calf serum.

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Cells were plated on glass hemocytometer cover glasses at a density of approximately 50,000 cells/cm<sup>2</sup> in 20 cm<sup>2</sup> plastic dishes. The following day, cells were treated with 0.2 µg/ml of mitomycin C (Sigma Chemical Co., St. Louis, MO) for 16 h and then fused with chick erythrocytes.

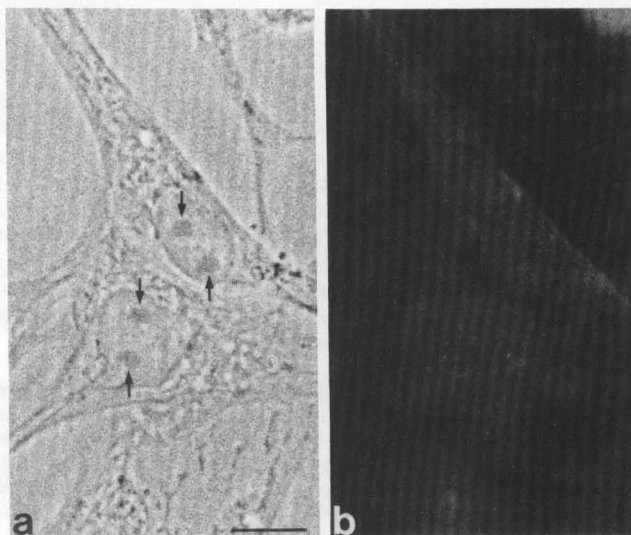


FIGURE 1 Cultured chick fibroblasts as seen with phase-contrast (a) and epifluorescence (b) optics after staining with antibodies to rat RNA polymerase I. Chick nucleoli are completely negative (denoted by arrows in a). Bar, 10 µm. × 1,000.

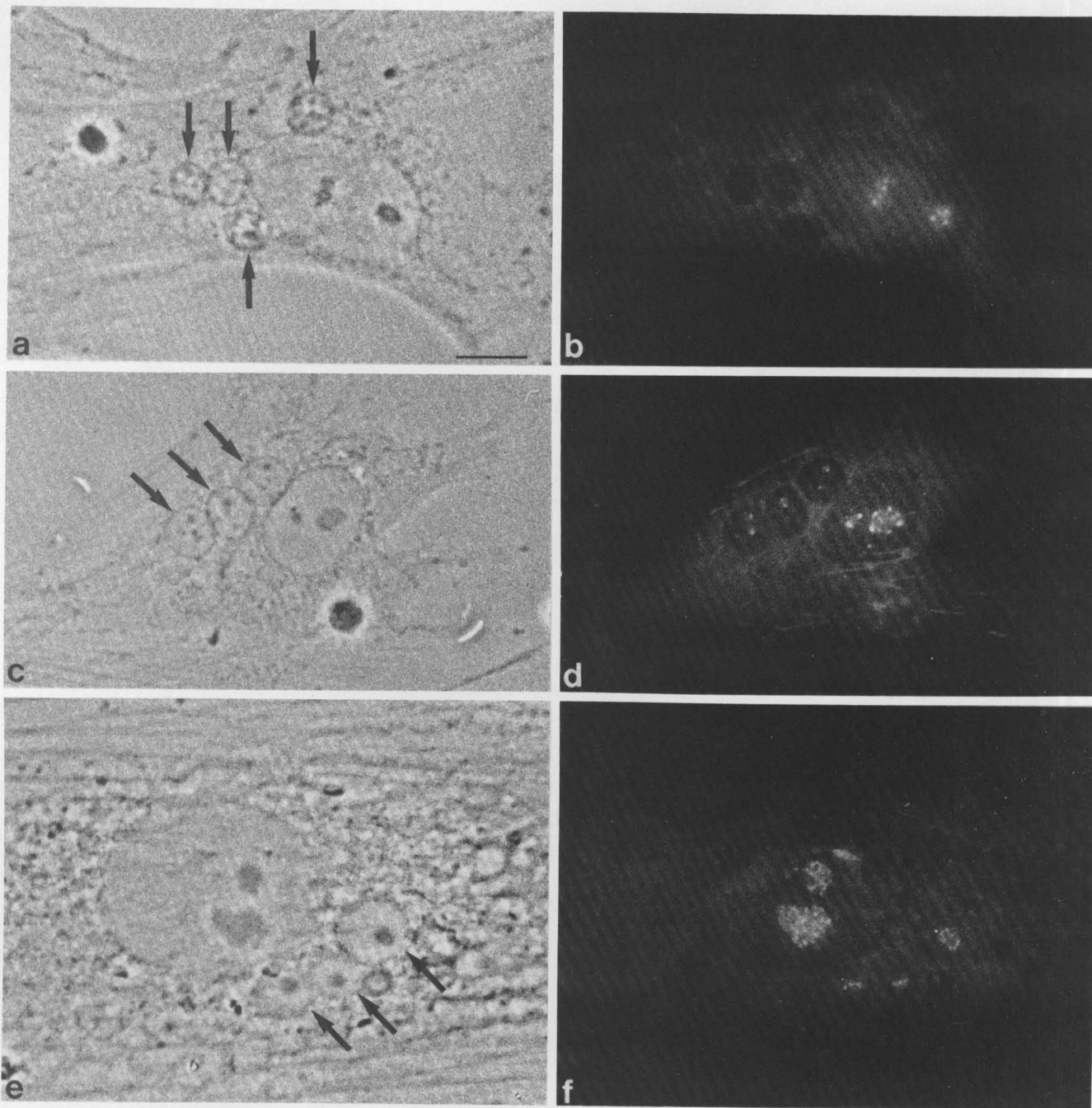


FIGURE 2 Rat myoblast  $\times$  chick erythrocyte heterokaryons processed for immunofluorescence microscopy using antibodies to rat RNA polymerase I are shown in phase contrast (a, c, and e) and epifluorescence (b, d, and f). (a and b) 28 h after fusion. Nucleoli present in unfused rat myoblasts and in the rat nuclei of heterokaryons are fluorescing in a punctate pattern while the chick erythrocyte nuclei (arrows in a), at this stage, are negative. (c and d) 72 h after fusion. Newly developed nucleoli of chick erythrocyte nuclei present in heterokaryons (arrows in c) are stained. (e and f) 190 h after fusion. Nucleoli have developed further in the chick erythrocyte nuclei of the heterokaryons (arrows in e). Several foci of polymerase I can be detected in some of the larger chick nucleoli, similar to the fluorescence pattern of the rat myoblast nucleoli. (a) Bar, 10  $\mu$ m.  $\times$  1,150. All micrographs are magnified to the same scale.

**Fusion:** Mitomycin-treated L6J1 monolayers on glass slides were washed extensively with PBS and fused with chick erythrocytes, with a 10-fold excess of erythrocytes over L6J1 cells using UV-inactivated Sendai virus (3). Excess erythrocytes were removed 20 h after addition of the virus by rinsing with PBS. Details of the fusion procedure have been reported elsewhere (3). Heterokaryon cultures were maintained in DME, 10% FCS.

**Fixation:** Slides were fixed at different time points 8–190 h after the start of the fusion. After rinsing in PBS, the slides were immersed in methanol at  $-20^{\circ}\text{C}$  for 5–10 min, dipped in acetone at  $-20^{\circ}\text{C}$ , and air-dried.

**Antisera:** Rabbit antiserum to RNA polymerase I from a rat hepatoma (9) was used to stain chick erythrocyte heterokaryons and, as controls, chick fibroblasts, cardiac myoblasts, and frozen sections through various chick tissues.

Antibody binding was detected with goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC; Miles-Yeda, Rehovot, Israel).

## RESULTS AND DISCUSSION

The RNA polymerase I antibodies used did not react with the nucleoli of cultured chick fibroblasts (Fig. 1, a and b) nor of cardiac myoblasts and of cryosectioned liver of chicken, but gave a distinct fluorescence with rat myoblast nucleoli (Fig. 2, a and b).<sup>1</sup> Thus, the antibodies were sufficiently species-specific to detect selectively rat RNA polymerase I in heterokaryons.



Chick erythrocyte nuclei in heterokaryons fixed at 28 h after fusion showed no fluorescence after incubation with antibodies to RNA polymerase I, whereas the rat nucleoli revealed finely punctate staining (Fig. 2, *a* and *b*). In a few heterokaryons fixed at 48 h after fusion a small number of chick nuclei showed single small fluorescent granules (data not shown). At 72 h after fusion, however, the majority of the erythrocyte nuclei contained one or two fluorescent bodies which coincided with small nucleolus-like structures observed in the phase-contrast microscope (Fig. 2, *c* and *d*). The correlation between nucleoli and antibody staining was even more obvious at 190 h after fusion (Fig. 2, *e* and *f*). At this point of time, virtually all chick erythrocyte nuclei have developed positively stained nucleoli. In those erythrocyte nuclei that contained large, well developed nucleoli, the fluorescence had a granular appearance similar to the pattern seen in the L6J1 nuclei present in unfused cells and in heterokaryons (Fig. 2, *a* and *b*) as well as in nucleoli of other mammalian species.<sup>1</sup>

Our results confirm previous immunological and biochemical studies (12) and show that the reactivation of chick erythrocyte nuclei is paralleled by a selective uptake of mammalian nucleus-specific components. While most of the earlier work was done with human nuclear autoantibodies of unknown biochemical specificity, the present study is the first to show that a defined enzyme of mammalian origin enters the reactivating chick erythrocyte nucleus and is concentrated in the newly formed nucleolus.

The time-point of the first detection of rat RNA polymerase I antigen in the chick nuclei by indirect immunofluorescence microscopy is markedly later than that observed for the mammalian nucleoplasmic antigens examined in previous studies (4, 5). The kinetics for the appearance of rat RNA polymerase I agree well, however, with the development of nucleolar structures and with the time-point of the first synthesis of chick-specific rRNA (13). The time and location of polymerase-I-antigenicity agree also with the appearance of  $\alpha$ -amanitin-resistant RNA polymerase activity (14) as detected by a cytochemical assay based on incubation with labeled nucleoside triphosphates and drugs (15). Using this type of assay as well as biochemical techniques, we recently demonstrated that rat RNA polymerase II enters the nucleoplasm of chick nuclei in rat-chick heterokaryons and is active in transcribing some of the chick globin genes (16). We conclude that during reactivation mammalian RNA polymerases of the types I and II migrate into the chick erythrocyte nuclei and become distributed in their respective subcompartments.

Recent evidence indicates that in heterokaryons rat RNA polymerase II is actively transcribing the chick genome (16). Our present results suggest but do not prove that rat polymerase of type I is actually transcribing the chick rRNA genes. Several observations support this notion. (*a*) RNA polymerase I activity is not detectable in mature avian erythrocytes, indicating the absence of this class of polymerase (17), in contrast to the presence of some residual RNA polymerase II activity (17, 18). Based on the results obtained by Harris et al. (13), it seems highly unlikely that synthesis of chick-specific polymerases takes place before the development of nucleoli since during this period few, if any, messenger RNA molecules are transferred from the chick nucleus into the cytoplasm of the heterokaryon. (*b*) In heterokaryons formed between HeLa cells and chick erythrocytes, nucleolar transcription is mainly dependent on a thermosensitive RNA polymerase activity

that is characteristic for the RNA polymerase I of human origin (19). (*c*) RNA polymerase I is capable, in vivo, to correctly transcribe heterologous rRNA genes as shown by injection of different kinds of rDNA into *Xenopus* oocyte nuclei (21, 22). (*d*) The cytochemical localization technique used in the present study detects only template-bound and not free forms of rat RNA polymerase I.<sup>1</sup> Thus, the immunofluorescence in the reformed chick nucleoli most likely reflects the presence of rat RNA polymerase I bound to the chick RNA genes that have initiated transcription, although we have not directly demonstrated this point.

The mechanisms by which RNA polymerases as well as other nucleus-specific proteins are selectively concentrated in defined nuclear compartments are not yet known. This phenomenon may be related to the affinity of the respective proteins for different chromatin conformations, the presence of signal sequences in the polypeptides and/or to special transport mechanisms (for a recent review, see reference 22). Chick erythrocyte heterokaryons may be a valuable system in which such mechanisms of nucleocytoplasmic sequestration can be studied in greater detail using specific antibodies to defined nuclear proteins.

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