Release of Particle Associated RNA Dependent DNA Polymerases by Primary Chicken and Quail Embryo Cells

Bauer, G.[1], Mattersberger, H., Soo, R., Jendis, J., Hofschneider, P.H.

1 present address: McArdle Laboratory for Cancer Research, Madison, Wisconsin 53706. USA
Max-Planck-Institut für Biochemie, Abt. Virusforschung. D-8033 Martinsried. Federal Republic of Germany

Introduction

Recently a new RNA dependent DNA polymerase has been described [1,2]. This enzyme is enclosed in sedimentable structures – "particles" –, and can be prepared from the allantoic fluid of embryonated virus free chicken eggs. The "particles" and the purified polymerase are different from the known avian RNA tumor viruses [1,2,3]. In this report we describe the appearance of this enzyme in the culture medium of primary chicken and quail embryo cells.

Results

Primary chicken and quail embryo cells release a particle associated RNA dependent DNA polymerase into the culture medium. The total amount of enzyme released is proportional to the number of cells plated. A maximum amount of enzyme is found in the medium when embryos of the age of six resp. eight days are used for culturing. The release after the onset of the cultures shows a well reproducible pattern with a peak around the third day and declines then within three to four days to background values. Secondary cultures release a significantly lower amount of enzyme. Tertiary or later cultures do not release detectable amounts of enzyme at all. In Fig. 1 the results obtained with primary chicken cells are shown. The experiments with primary quail cells led to almost identical results.

To characterize the enzyme, particles were purified from culture medium and the enzyme was tested for the ability to transcribe homo- and heteropolymeric RNA (Table 1). Table 1 clearly shows that the enzyme catalyzes RNA dependent DNA synthesis in the presence of an appropriate template primer complex. No endogenous DNA synthesis could be detected under the conditions used. When globin mRNA-oligo dT was used as a template primer complex the product was shown to consist of DNA with a base sequence complementary to globin mRNA.

The enzymes have further been characterized by determining the optimum conditions for reverse transcription of homopolymeric RNA. As can be seen from Table 2 both the enzymes have nearly identical optimum assay conditions and they are in this respect somewhat different from the respective enzyme of the Avian Myeloblastosis Virus (AMV). These differences be-

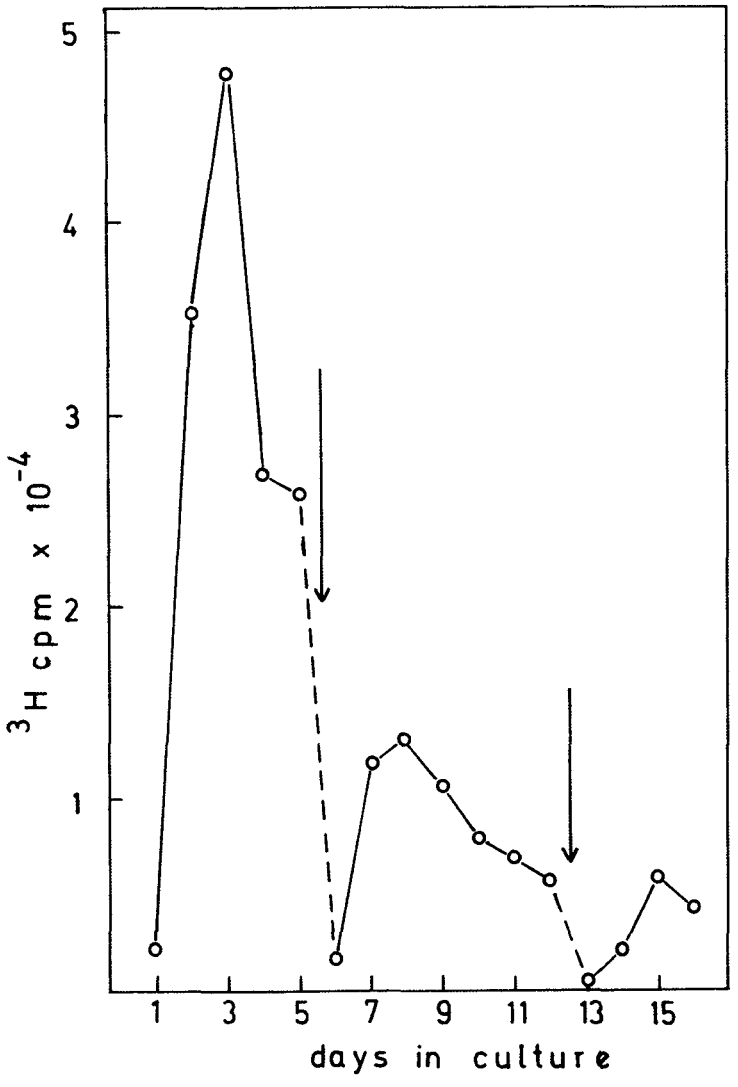

Fig. 1. Release of a particle bound DNA dependent DNA polymerase by primary, secondary and tertiary chicken cell cultures. Primary cells were plated at a density of 10×10^6 cells/plate; after 5 and after 12 days cultures were passaged (marked by arrows) and seeded at 5×10^6 cells per plate. Enzyme measurement as described in [4]

come more pronounced when the optimum conditions are studied in more details [2,5]. A distinct difference is found in inhibition experiments with IgG directed against the AMV-enzyme. In contrast to the viral enzyme the enzymes from primary quail and chicken cell cultures are not or only weakly inactivated under identical assay conditions. To obtain 50% inhibition of the "chicken enzyme" a 40–200 fold excess of IgG compared to the homologous AMV enzyme must be added [1,4], whereas in the case of the "quail enzyme" no inhibition could be observed (Fig. 2). In contrast to that, the polymerases from Rous Associated Virus O (RAV-O), an endogenous Avian Leucosis Virus (ALV) as well as polymerases from exogenous ALV/ASV are inhibited to the same extent as the AMV-enzyme [5]. This result shows that 1. all the polymerases within the ALV/ASV group behave identical in this assay and are 2. different from the enzymes described here.

The chicken- and quail-enzymes can also be distinguished from the AMV-enzyme when the enzymes are incubated at high temperatures (52°) with or

Table 1. Reverse transcription of heteropolymeric RNA by particle associated reverse transcriptase released by chicken and quail cells

Template	Primer	Conditions	cpm ^{3}H-dGMP incorporated	
			a	b
globin mRNA	$(dT)_{12}$	complete	5000	819
globin mRNA	$(dT)_{12}$	complete + RNAse	270	n.t.
globin mRNA	—	complete	190	93
70S AMV RNA	—	complete	220	n.t.
70S AMV RNA	$(dT)_{12}$	complete	2340	n.t.
poly (C)	$(dG)_{12-18}$	complete	113700	11300

The reaction mixture contained as described in the standard polymerase assay for reverse transcription of homopolymeric RNA (1): Magnesium acetate (10 mM). KCl (40–80 mM). Tris-HCl pH 8.3 (50 mM). dithioerythritol (10 mM). ATP (1 mM). BSA (50 µg/100 µl). template primer complex (1 µg). except that ^{3}H-dGTP (19 Ci/mMole) was present at a concentration of 26 µM (50 µCi per 100 µl assay).

The assays for reverse transcription of heteropolymeric RNA contained 3 µg RNA and 1 µ $(dT)_{12}$ respectively and unlabelled dATP. dCTP and dTTP at concentrations of 0.3 mM. When particle-bound polymerase was tested. the assay contained 0.1% NP40. The reaction volume was 100 µl. The incubation was at 37°C for 35 minutes for the chicken enzyme (a) and 45 minutes for the quail enzyme (b). Incorporation was determined as TCA precipitable counts in 30 µl (a) and 100 µl (b) resp.

Table 2. Different optimum conditions for AMV- and particle-polymerases

Enzyme from	Mg^{2+} (mM)	Mn^{2+} (mM)	K^+ (mM)	pH	Temp. (°C)	K_M-dGTP (µM)
AMV	10	0.8	80	8.5	40	3.3; 20[a]
Quail cells	5	0.5	60	8.5	45	4.0; 28[a]
Chicken cells	5	0.4	60	8.5	45	25

[a] biphasic Lineweaver-Burk plot

Standard polymerase assay conditions were as described in Table 1 with poly (C) $(dG)_{12}$ as template primer complex at a concentration of 1 µg per 100 µl assay and ^{3}H-dGTP (19 Ci/mMole) at a concentration of 2.6 µM (5 µCi per 100 µl assay).

without 70S AMV RNA (Table 3). They are significantly more stable at high temperatures compared to the AMV polymerase. In the presence of 70S RNA, AMV polymerase is markedly protected against heat denaturation; no such stabilizing effect of 70S RNA is detectable with the enzymes prepared from cell culture medium.

The chicken- and quail-enzymes are also different from the polymerase of the Reticuloendotheliosis virus (REV). As shown in Table 4 they prefer as cation Mg^{2+} instead of Mn^{2+} as does the REV enzyme. The ratios are 7,2, 4,6 and 0,4 resp.

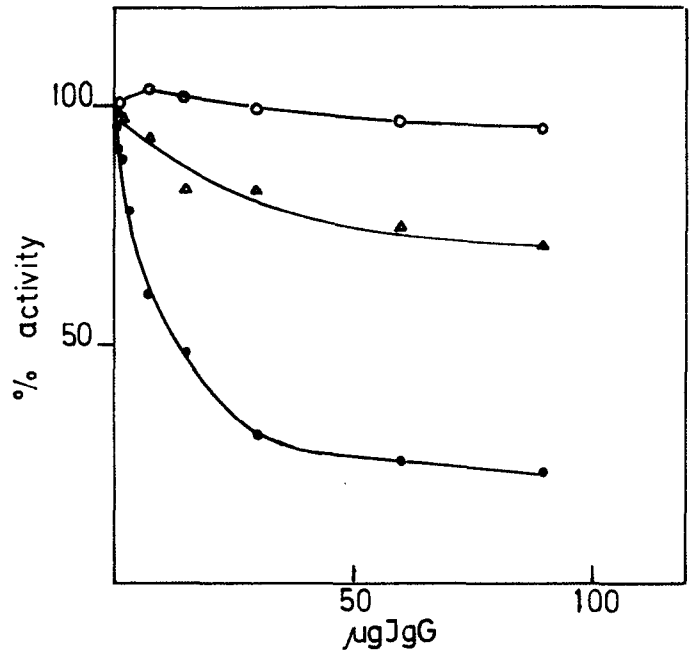

Fig. 2. IgG inhibition test of RNA dependent DNA polymerases from chick and quail embryo cell cultures. compared to AMV polymerase. Constant amounts of enzyme were preincubated with increasing amounts of IgG directed against AMV polymerase. Experimental details are as described in [4]. except that the incubation was carried out at 37°C for 15 min instead of 0°C for 15 h. This modification leads to a less rapid and less complete inactivation of the AMV enzyme than reported in [4]. but the activity of the quail-enzyme even in absence of IgG is better conserved

Table 3. Thermal inactivation of different reverse transcriptases with and without 70S AMV RNA at 52°C

Enzyme from	Time for 50% inhibition (min.) – 70S	Time for 50% inhibition (min.) + 70S
AMV[a]	0.8	4.7
Chicken cells	12	12
Quail cells	19	20

[a] Avian myeloblastosis virus

The enzymes were preincubated with and without 70S AMV RNA at 52°C for varying times and the residual activity determined in a standard polymerase assay with poly (C) $(dG)_{12}$ as template primer complex.

Table 4. Different cation preference of REV and particle polymerases

Enzyme from	Mg^{2+} ^3H cpm	Mn^{2+} ^3H cpm	Relation of activities Mg^{2+}/Mn^{2+}
REV[a]	478×10^3	1080×10^3	0.44
Quail cells	2.7×10^3	0.6×10^3	4.62
Chicken cells	13.0×10^3	1.8×10^3	7.22

[a] Reticulo endotheliosis virus

Standard polymerase assay conditions were. as described in Table 1. using 10 mM Mg^{2+} and 0.4 mM Mn^{2+} resp. Poly (C) $(dG)_{12}$ was used as template primer complex at a concentration of 1 µg per 100 µl assay and ^3H-dGTP (19 Ci/mMole) at a concentration of 2.6 µM (5 µCi per 100 µl assay).

No difference, however, is detected between the chicken-enzyme and the particle bound polymerase isolated from the allantoic fluid of embryonated chicken eggs [1]. For all properties tested [1,2,3,7] identical values were found.

Conclusions

These results show that primary chick embryo cells in culture release a particle bound RNA dependent DNA polymerase into the culture fluid. The enzyme is also found in the allantoic fluid of embryonated chicken eggs. This phenomenon is not restricted to the chicken system because primary quail embryo cells which are not known to express the so far known endogenous viruses release a particle bound RNA dependent DNA polymerase as well. In both cases this enzymes may be part of an hitherto unknown endogenous viral system. However, since the expression of the chicken- and quail-enzyme goes through a sharp peak during embryogenesis one may speculate on a physiological role for the newly described enzymes during development.

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