The in vitro Translation of Rous Sarcoma Virus RNA and Function of the Viral Protein During the Viral Replication

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Abstract

The *gag* gene and *pol* gene of the Rous sarcoma virus are translated *in vitro* from the 35S viral RNA. The *env* gene cannot be translated *in vitro* from the 35S RNA. For the *in vitro* translation of the *src* gene. 3' end fragments of the viral RNA are used.

The *gag* protein p15 has a proteolytic activity and specifically processes its own protein precursor pr76. The *gag* protein p19 suppresses the *in vitro* translation of the *pol* gene.

The replication of RNA tumor viruses was thought to be strictly dependent on their host cell activities. There is recent evidence, however, that some parts of the replication of avian RNA tumor viruses are independent of their host functions; they are controlled by a feedback mechanism of their viral gene products.

Which viral proteins are involved and what are their functions?

In order to determine which viral proteins have functions during the replication cycle, a cell-free system was used to synthesize the viral proteins; a) to prove that those proteins are in fact coded by the viral genome, b) to work in a system which is free of host cell contamination.

In a cell free system from mouse ascites Krebs II cells the 35S RNA of Rous sarcoma virus (RSV) has been translated into a precursor of the viral group specific antigen *(gag)* protein, pr76 [1], and a precursor of the reverse transcriptase *(pol)*, pr180 [2]. Both protein precursors are not processed when synthesized in vitro, even after long incubation periods. Upon addition of *gag* protein p15 however (which is purified in 6,0 M GuHCl on an agarose column) the precursor pr76 is cleaved within 30 min into the *gag* proteins p27, p15, p12 and pr32 which is a protein precursor to p19 (Fig. 1) [3].

This cleavage is very similar to that observed in the RSV infected cells. Fig. 2 shows that the *pol* precursor pr180 is cleaved as well by the *gag* protein p15 [2].

These results indicate a new way of how precursors can be processed: By a specific cleaving activity which is contained in one of their cleaved end products. Since our results were obtained in vitro, a question arises:

Is this in vitro cleavage an artefact or does p15 play a physiological role in the intracellular processing?

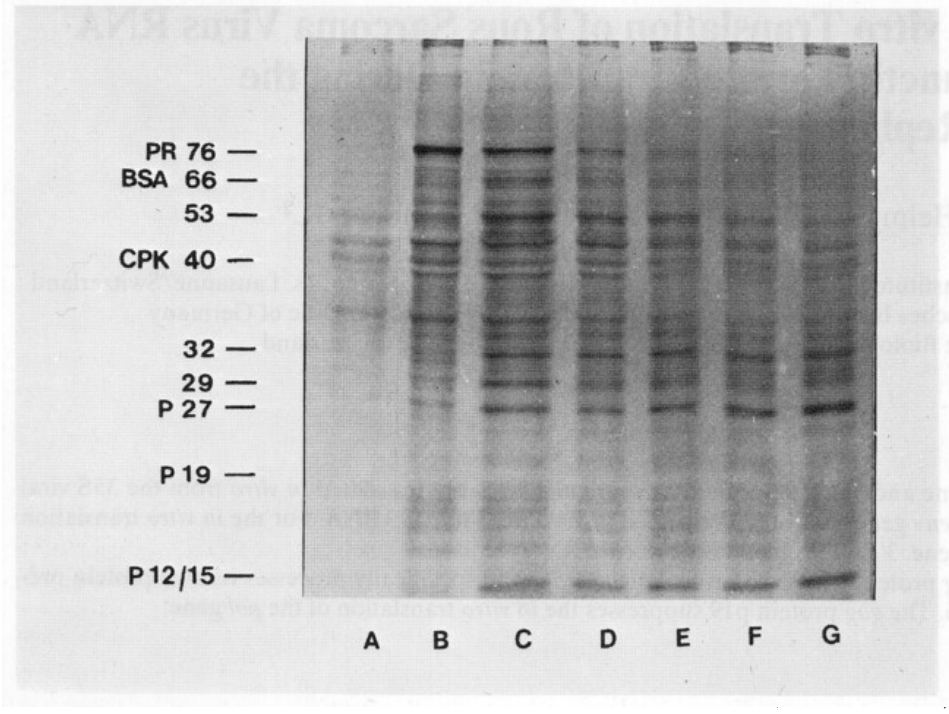

Fig. 1. In vitro processing of pr76 by p15. Autoradiograph of SDS-gel electrophoresis of ^{35}S-methionine labeled polypeptides synthesized in a cell free system directed by 35S RSV RNA (1.5 µg/20 µl). (A) no RNA added, (B) plus RNA, synthesis for 60′ (C–G) further incubation with unlabeled methionine in presence of p15 for (C) 5 min, (D) 10 min, (E) 15 min, (F) 30 min, (G) 4-fold amount of p15 was added and incubated for 10 min

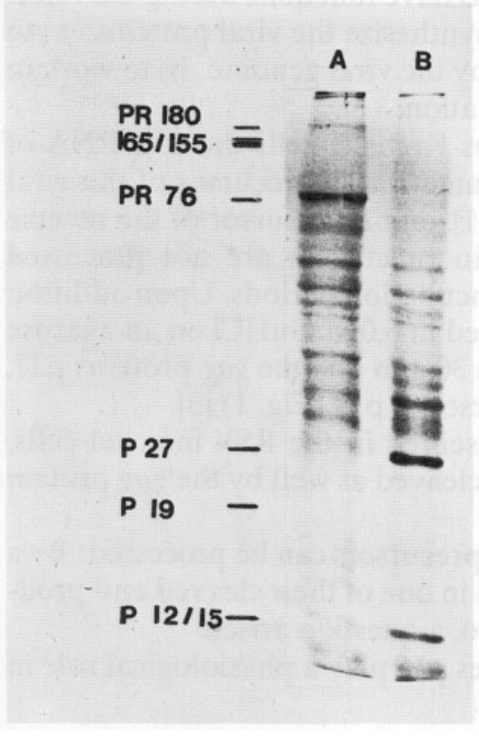

Fig. 2. Autoradiograph of in vitro products as in Fig. 1 (A) incubation for 90 min (B) further incubation for 30 min with unlabeled methionine in presence of p15

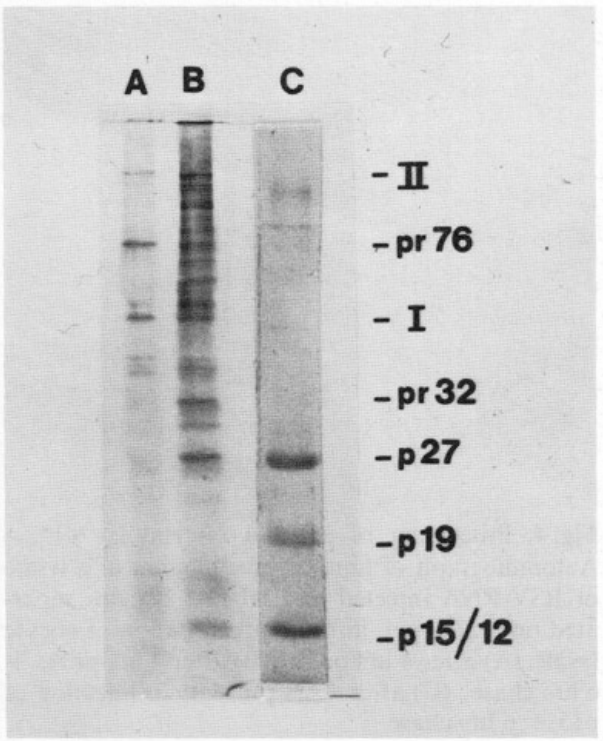

Fig. 3. Intracellular processing of pr76 by fusion-injection of p15. – Autoradiograph of immunoprecipitation of ^{35}S-methionine labeled hamster cell lysate separated on SDS-gel. (A) Mock injected cells (B) p15 injected cells (C) viral markers

The answer is given by the following two experiments:

a) Purified protein p15 was injected into a RSV transformed hamster [4] cell line which is not permissive for RSV replication but contains small amounts of uncleaved *gag* precursor pr76. The injection was carried out by the fusion-injection technique [5] in which p15 is loaded into erythrocytes by a minor osmotic shock and those loaded erythrocytes are then fused to ^{35}S-methionine labeled hamster cells [6]. After two hours of incubation the cells had been lysed and anti-*gag* serum added. Processed *gag* proteins had been precipitated from the p15 injected cell lysate (not from the mock-injected control cells (Fig. 3) which shows that pr76 has in fact been intracellulary processed by the protein p15 [6].

b) A further experiment in oocytes confirms the intracellular cleavage by p15. The *gag* precursor pr76 can be synthesized in frog oocytes upon injection of 35S RSV RNA. The precursor is consecutively very slowly processed within the oocytes and the cleavage is completed after 72 hrs [7]. – If p15 is involved in the cleavage, the reaction time should be significantly decreased by injecting p15 in the pr76 containing oocytes: In our experiment oocytes had been injected with RSV RNA; precursor pr76 was synthesized during a 4 hrs pulse with ^{35}S-methionine after injection (Fig. 4A), the 4 hrs pulse was succeeded by a 6 hrs chase: almost no cleavage was detectable; the pulse was succeeded by injection of p15, and a 6 hrs chase: most of the precursor pr76 is cleaved into the *gag* proteins (Fig. 4C) as it

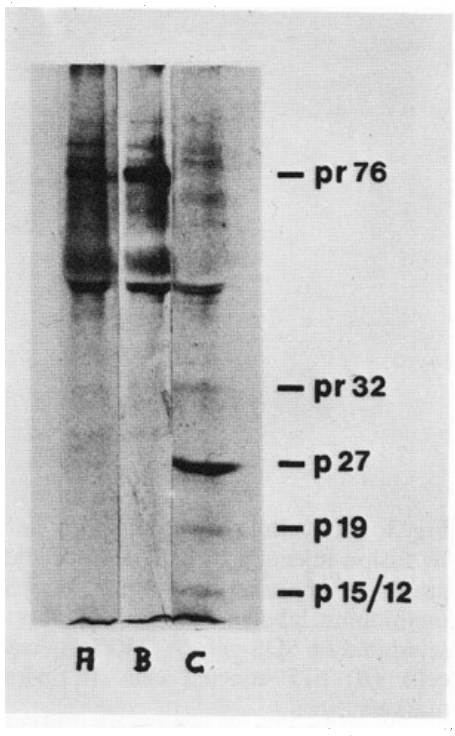

Fig. 4. Processing of pr76 in oocytes by p15. – Autoradiograph of immunoprecipitation of a lysate of RSV RNA injected and labeled oocytes separated on a SDS-gel. Immunoprecipitation of oocyte lysate (A) after 4 hrs pulse, (B) after 4 hrs pulse + 6 hrs chase. (C) after 4 hrs pulse, then injection of p15 + 6 hrs chase

had been found by immunoprecipitation with anti *gag* serum (von der Helm, K., and Rungger, D., manuscript in prep.).

Conclusion: RSV *gag* protein p15 is involved in both in vitro and in vivo processing of the *gag* precursor protein pr76. The processing of pr76 is essential for the assembly and thus for the replication of the virus. –

Furtheron we studied viral proteins other than p15 for possible involvement in the viral replication and made an interesting observation: the *gag* proteins p12 and p19 are known to bind specifically to the viral RNA [8,9]. When p12 is added to a cell free system, the translation of the RSV RNA is almost completely suppressed. When p19 is added to such an in vitro system, the translation of only the *pol* gene is inhibited, i.e. the synthesis of pr180 is specifically suppressed while the pr76 precursor is synthesized (Fig. 5).

In infected cells about 10 times less pr180 precursor than pr76 precursor is found. We suggest that the *gag*-protein p19 controls the synthesis of the pr180 precursor during viral replication. –

The *gag* and *pol* gene, and not the *env* nor *src* gene can be translated in vitro from the 35S RSV RNA. However, RNA fragments of the 3′ end of the viral RNA, separated into two size classes of about 15–20S and 20–22S, both containing the *src* gene, could be translated in vitro [10,11,13].

The translation products of the 15–20S RNA are polypeptides of 25, 35 and 43K dalton and the translation product of the 20–22S RNA is predominantly a polypeptide of 60K dalton. Rabbit antiserum against the 60K polypeptide [12] (a kind gift of R. Erikson) precipitates the 60K polypeptide in vitro made from the 20–22S RNA (Fig. 6B) [13] and from the 15–20S RNA predominantly the 25K and to a lesser amount the 35K and 43K polypeptides (Fig. 6C). The 3′ end RNA fragments from a transformation defective (td)

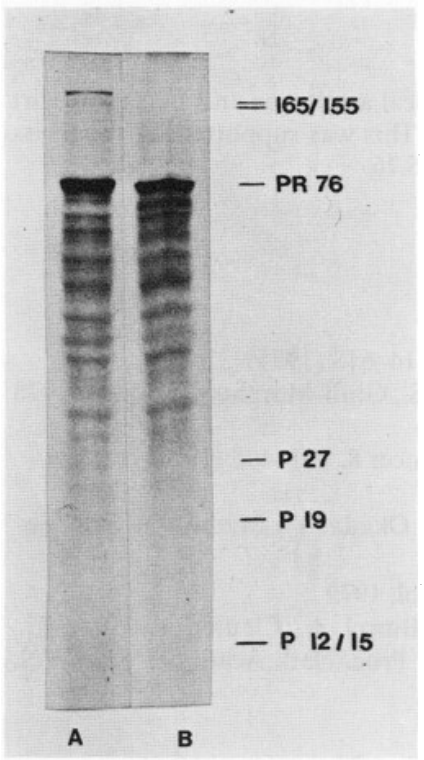

Fig. 5. Autoradiograph of in vitro products as in Fig. 1. In vitro translation of 35S RSV RNA in absence (A) and presence (B) of protein p19

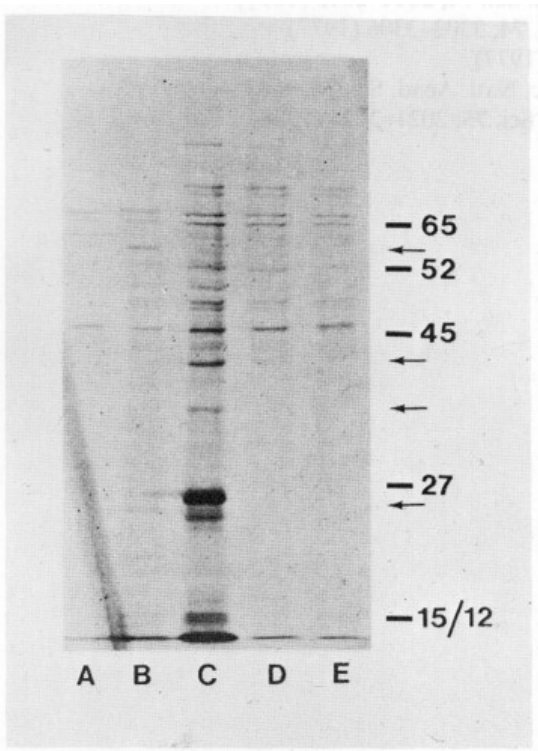

Fig. 6. Autoradiograph of immunoprecipitation of in vitro translation of 3′-end src-containing fragments of RSV-RNA: (B) 24–20S (C) 20–15S, and (td) RSV RNA: (D) 24–20S (E) 20–12S. (A) endogenous background of in vitro system. All samples are immunoprecipitated with antiserum against src product

mutant of RSV which has a deletion in src, were not translated in vitro (Fig. 6D and E) implying that the in vitro made polypeptides are in fact *src* gene products. The functions of the smaller polypeptides are not known. It is implied that the *src* gene product plays a role during the transformation of cells. Collett and Erikson found that the 60K polypeptide synthesized in vitro as well as in vivo have a protein kinase activity [14].

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