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Proteolytic Processing of Avian and Simian Sarcoma and Leukemia Viral Proteins

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A. Introduction

Viral proteins of avian and mammalian leukemia and sarcoma viruses are synthesized in their host cells primarily as precursors. They are successively processed to smaller proteins, which then can be assembled into virus particles (Vogt et al. 1975; Arcement et al. 1976; Okasinsky and Velicer 1976; Eisenman and Vogt 1978). This processing has been found to be caused by a virus-specific proteolytic enzyme. The protease p15 is coded by the genome of leukemia/sarcoma viruses; it is part of the internal viral core, the group-specific antigen (gag) protein, and cleaves its own gag precursor (von der Helm 1977). This precursor cleavage can be demonstrated in vitro with purified p15 from AMV and gag precursor bound to an antibody (directed against gag proteins) and Staph. aureus protein A (Dittmar and Moelling 1978).

Using this system we studied whether protein precursors with gag sequences of other related or unrelated oncornaviruses could be cleaved by the avian gag protein p15.

This paper describes the cleavage of a 110 k dalton fusion protein of the replication-defective avian acute leukemia virus MC 29 (Langlois et al. 1967; Bister et al. 1977) and a gag protein precursor pr65 of Simian sarcoma virus (SSV) (Wolfe et al. 1971; Deinhardt et al. 1978) by avian p15 into discrete smaller proteins. Since protease p15 evidently cleaves off the gag sequences of the fusion protein gag-x (whereas the x sequences may represent the putative "onc"-gene sequences), we discuss how p15 could be used as a tool for mapping transformation proteins.

B. Results and Discussion

To determine the optimal conditions for cleavage of IgG-bound pr76 by the p15 protease, the immunocomplex containing ^{35}S-methionine-labeled pr76 was incubated for different periods of time and different salt concentrations in presence of p15 protease (B. Konze-Thomas and K. von der Helm, to be published). Protease p15 was isolated from virions by conventional methods (von der Helm and Konze-Thomas 1980; B. Konze-Thomas and K. von der Helm, to be published). Figure 1 shows that during 30 min of incubation in 0.15 M NaCl processing of pr76 and pr180 commenced and is completed after 6 h. In presence of higher salt concentration (i.e., 1 M NaCl) in vitro processing is already completed after 2 h of incubation. Evidence for the completion of the processing is the disappearance of the polypeptide pr32 which is an intermediate precursor to p19 (Vogt et al. 1975; von der Helm 1977). Cleavage of other non-gag-containing polypeptides (i.e., gp85) or further cleavage of the processed gag proteins (p27, etc.) has never been observed, even with very long incubation periods (not shown).

I. Cleavage of MC 29 Protein 110 k by p15

The defective avian acute leukemia virus MC 29 has among other defects a deletion of the p15 gene (Bister et al. 1977; Mellon et al. 1978). A 110 k dalton protein, the only virus-specific polypeptide synthesized in infected and transformed nonproducer cells is not processed. It is a fusion protein of an incomplete gag gene (p19 and p27) and unknown

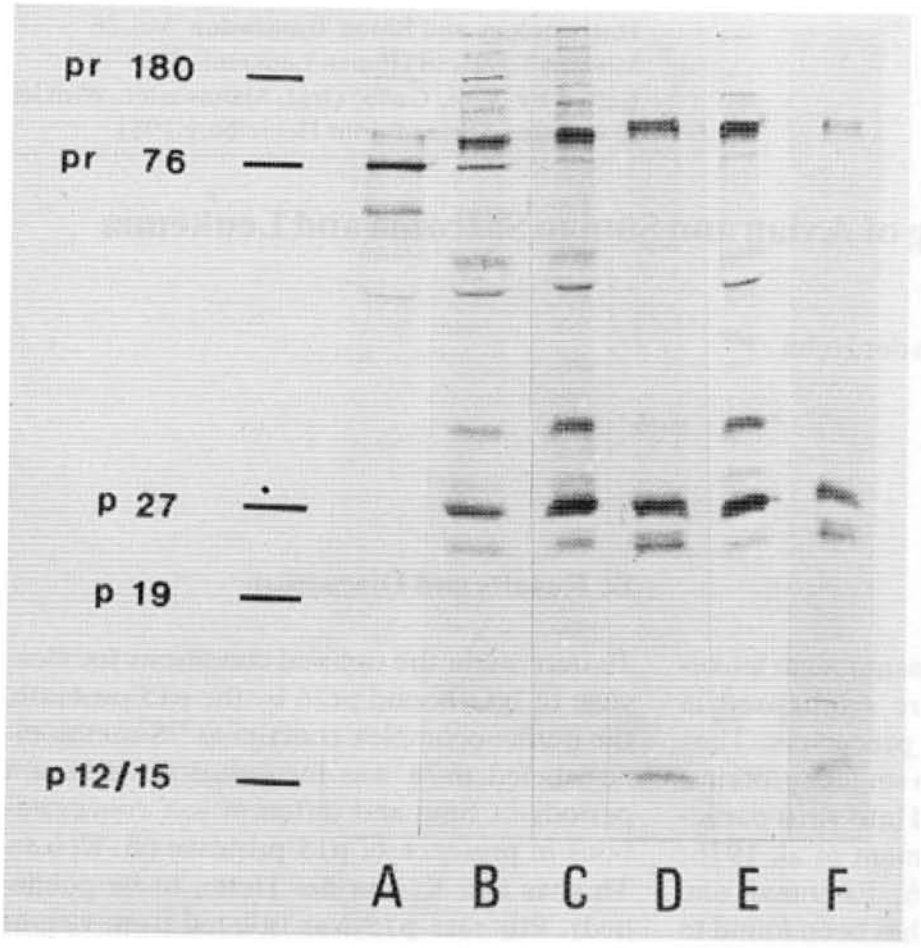

Fig. 1. In vitro cleavage of pr76 by p15. RSV-infected chicken fibroblasts were labeled for 1 h with ^{35}S-methionine, lysed, and immunoprecipitated by anti-p27 serum and protein A sepharose (B. Konze-Thomas and K. von der Helm, to be published) *(A)*, untreated control or incubated in 0.15 *M* NaCl at 37°C with 0.7 ug of protease p15 for ½ h *(B)*, 2 h *(C)*, 6 h *(D)* or for 2 h in 0.5 *M* NaCl *(E)* or 1 *M* NaCl *(F)*. After incubation the samples were analysed by 12.5% SDS-PAGE and autoradiography

sequences coding for a putative transformation protein (Dittmar and Moelling 1978).

We used the p15 protease as a tool for restrictive protein cleavage in vitro and tried to cleave off the gag sequences in order to determine the size of the non-gag sequences of the 110 k dalton polypeptide. From a ^{35}S-methionine-labeled lysate of nonproducer cells, the 110 k d protein had been immunoprecipitated by a serum directed against p27 structural protein of avian leukemia/sarcoma virus (B. Konze-Thomas and K. von der Helm, 1980, to be published). After incubation of the immunocomplex with p15 the 110 k dalton protein had been cleaved into proteins of the molecular weight 75 k, 55 k, 32 k and 25/24 k dalton (Fig. 2). A comparison of these proteins by a V8 protease digestion analysis suggested that all of them derived from the 110 k dalton protein (not shown). By this analysis the 75 k d polypeptide appears to be an intermediate precursor to the 55 k d product. The 25/24 and 32 k d polypeptides react strongly with anti-gag serum, the 55 k d polypeptide, very weakly. We assume that the 24/25 k d and 32 k d polypeptides are gag-related proteins, while the 55 k d polypeptide contains no gag sequences and may represent the putative "onc" part of the fusion protein.

II. Immunoprecipitation of Intracellular SSV-Specific Proteins

Simian sarcoma virus was originally isolated from a spontaneous sarcoma of a woolly monkey (Wolfe et al. 1971). Similar to the MC 29 virus, this virus transforms cells and is replication-defective. Isolates of this virus always contain excess helper virus (SSAV) (Wolfe et al. 1971). Cells infected solely with SSV do not produce virus. We immunoprecipitated ^{35}S-methionine-labeled lysates of several SSV nonproducer or SSV (SSAV) producer cells with serum against the SSAV viral gag

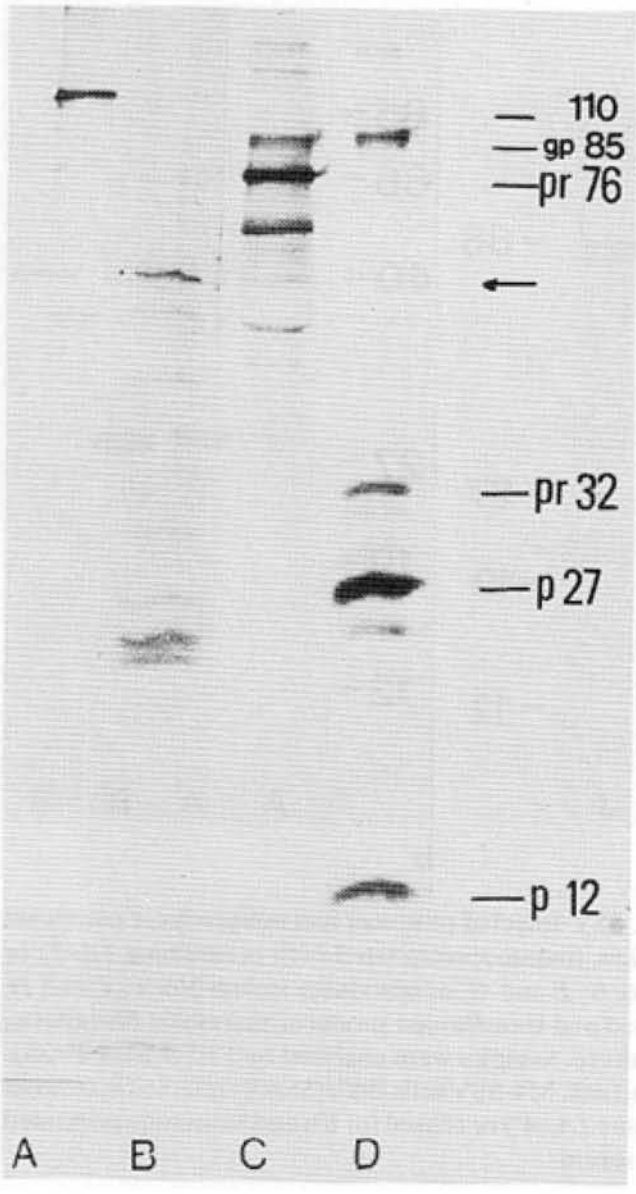

Fig. 2. In vitro processing of the MC 29 polypeptide 110 kd. Quail cells, nonproductively infected by MC 29 (8, kindly provided by K. Bister), were radioactively labeled, immunoprecipitated *(A)* and cleaved by p15 *(B)* as in legend Fig. 1, (E). As control, ^{35}S-methionine labeled RSV infected chicken fibroblasts *(C)* were processed in vitro by p15

protein p30 (B. Konze-Thomas and K. von der Helm, to be published). Two major virus-specific polypeptides could be detected (Fig. 3a) within the different cell lines: the p30 gag protein and a polypeptide of 65 k dalton.

By a pulse chase experiment we investigated the question whether the 65 k d polypeptide is a precursor protein to p30. It can be seen from Fig. 3b that after a 6 h chase the radioactivity had been chased obviously into the p30 protein (because of the 3-h pulse labeling period some p30 appeared already in the immunoprecipitate of the pulse).We assume the 65 k d polypeptide (pr65) to be the precursor to p30.

Since the pr65 appeared in producer as well as in nonproducer cells, it might possibly be a fusion protein like the MC 29 protein containing some gag sequences plus sequences for a "transformation" protein. In order to characterize this aspect of the 65 k d polypeptide we used the protease p15 again as a tool for "mapping" cleavage.

Figure 4 shows the cleavage of the SSV protein 65 k d by protease p15 in the presence of different salt concentrations: using 0.5 M NaCl, two proteins appear, one comigrating with p30 and another polypeptide of approximately 33 k dalton that did not immunoprecipitate with anti-p30 serum at the pulse chase experiment. It is, however, possible that this 33 k d polypeptide is an intermediate precursor protein to other non-p30 gag proteins. Alternatively this protein might represent a non-gag portion of the 65 k dalton protein. During the course of this meeting evidence has been gathered by others (see Bergholz and Thiel, this volume), that this described 65 k dalton polypeptide might contain exclusively gag sequences. If so, it has to be elucidated why a polypeptide containing pure gag sequences is synthesized in a nonproducer cell.

The transforming gene product of avian sarcoma virus exhibits protein kinase activity (Brugge and Erikson 1977). There is also evidence that transformation-specific proteins of other viruses like Abelson leukemia virus (Witte et al. 1980) exhibit protein kinase activity as well. We investigated the 110 k dalton protein of MC 29 and the 65 k dalton protein of SSV (SSAV) for kinase activity by in vitro labeling with ^{32}p-ATP but could not find evidence for it (data not shown). Recently, however, evidence was presented (Bister et al., see this vol.) that the 110 dalton protein of MC 29 comprises kinase activity.

The pp60^{src} protein of avian sarcoma virus and some other virus specific transformation proteins are phosphorylated either by autocatalytic action or other kinases. We labeled RSV-, MC 29-, or SSV-infected cells for 2 h with ^{32}P-orthophosphate and immunoprecipitated the cell lysates with the respective sera. In addition, an aliquot of each immunoprecipitate had been subjected to processing by p15 protease. The results are shown in Fig. 5. The pp60^{src} (besides pr76) of RSV was phosphorylated and not cleaved by p15. From lysates of MC 29 polypeptides of 110 k, 90 k, and 75 k dalton were phosphorylated. Upon

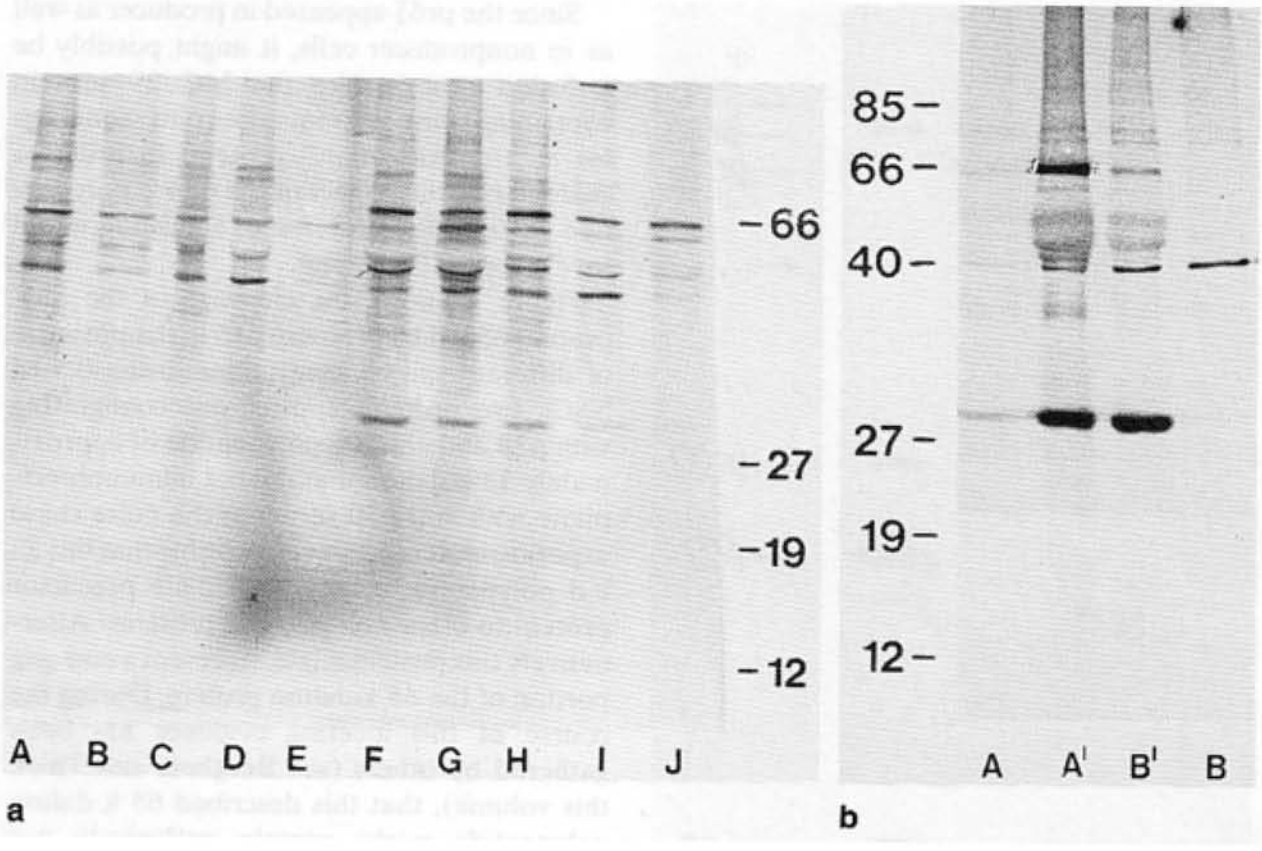

Fig. 3. a Immunoprecipitation of SSV specific proteins. SSV-infected producer and nonproducer marmoset (HF) cell lines were labeled for 4 h with ^{35}S-methionine, immunoprecipitated with preimmune *(A–F)* or anti-p30 serum *(F–J)*. *A* and *F*, nonproducer isolate No. 6; *B* and *G*, nonproducer isolate No. 1; *C* and *H*, producer No. 19; *D* and *I* M4-SSV, SSV(SSAV) infected and transformed producer marmoset fibroblasts; *E* and *J*, as producer No. 19, but grown in suspension culture. Samples were analysed by 12.5% SDS-PAGE and autoradiography. **b** Pulse-chase label of SSV-infected cell. M4-SSV cells [SSV(SSAV) infected marmoset fibroblasts] were pulse-labeled for 3 h with ^{35}S-methionine *(A, A″)* or chased for 6 h and immunoprecipitated *(B, B″)*. *A* and *B*, pre-immune serum; *A″ B″*, anti-p30 serum

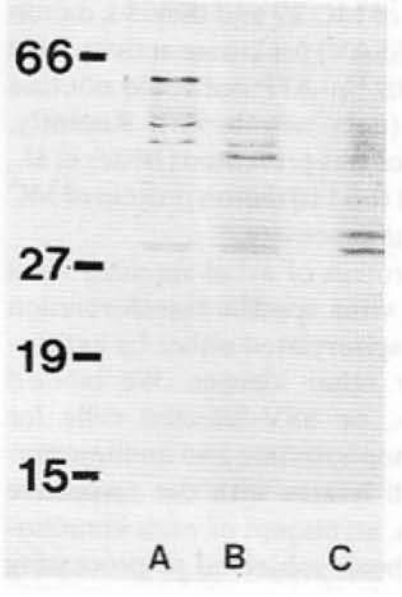

Fig. 4. Cleavage of SSV protein precursor pr65 by avian virus protein p15. M4-SSV cells were labeled and immunoprecipitated as in legend Fig. 3a *(A)* and aliquots subjected to p15-cleavage overnight in presence of 0.15 *M* NaCl *(B)* or 0.5 *M* NaCl *(C)*

addition of p15, the 75 k d polypeptide remained uncleaved, the 110 k d and 90 k d protein could be cleaved, and the radioactive label was found in two bands of about 70 and 64 k dalton and a 32 k d polypeptide (which is probably the intermediate precursor to the phosphorylated p19). However, no label could be detected in the range of 55 k dalton. We conclude that the 90, 70, and 64 k d polypeptides are intermediate precursors, that the 55 k d polypeptide is one cleavage end product which is not phosphorylated, and one of the other end products, the p32 (resp. p19), carries the phosphoryl rest.

The SSV(SSAV) 65 k dalton protein was phosphorylated. However, upon addition of p15 no significant shift of the phosphorylated 65 k d band could be detected. Further investigations now have to reveal whether this phosphorylation is implicated in an activity of a putative transformation gene product.

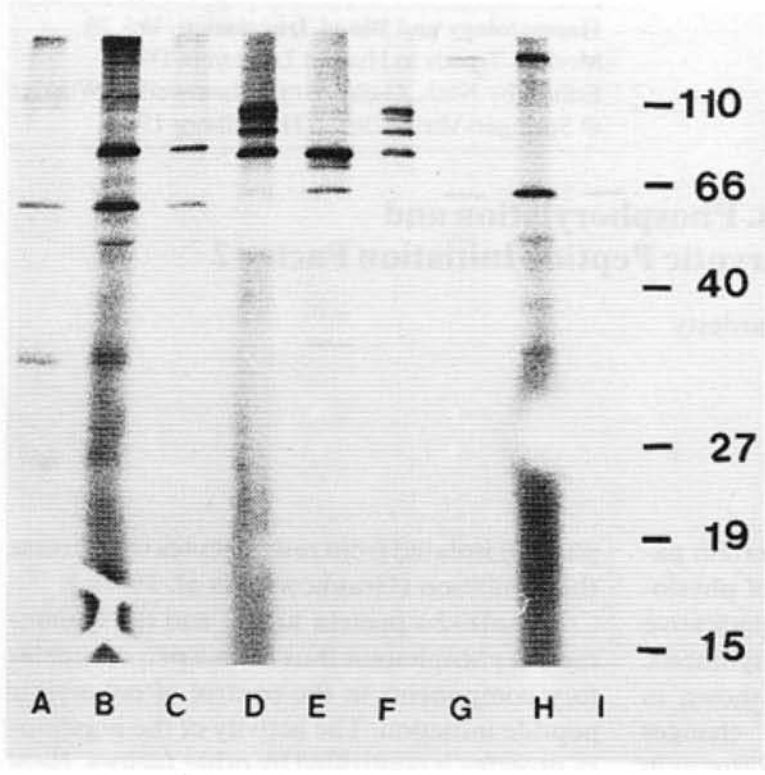

Fig. 5. Label of transformed cells in vivo by ^{32}P-orthophosphate and p15 cleavage. RSV-infected chicken fibroblasts *(A–C)*, MC 29 nonproductively infected quail cells *(D–F)*, and SSV(SSAV)-infected marmoset fibroblasts *(G–I)* were labeled with 1 uCi/ml ^{32}P-orthophosphate for 2 h, lysed, immunoprecipitated *(B, D, H)*, or subjected to an overnight incubation at 37°C in presence of p15 *(A, E, G)* or without p15-protease *(C, F, I)*. The samples were analysed by SDS-PAGE followed by autoradiography

Acknowledgments

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