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The Transformation-Specific Protein pp60^{src} from an Avian Sarcoma Virus

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A. Abstract

We have detected the avian sarcoma virus (ASV) transforming protein pp60^{src} in RNA tumor virus particles and used them as a source for its isolation. The partially purified protein has a molecular weight of 60K, exhibits protein kinase activity, and is indistinguishable from its cellular counterpart. It is released from the virus by nonionic detergent. The soluble molecule easily undergoes transition to a degraded form of 50/52K. Both 60K and 50/52K forms phosphoylate themselves and reveal protein kinase activity. Embedded in the viral membrane, pp60^{src} faces the inner coat, since it is inaccessible in intact virus particles to surface iodination, antibodies, and proteases.

B. Introduction

The transforming gene product of ASV, pp60^{src}, was found in transformed cells by means of antibodies which have been prepared in tumor bearing rabbits (TBR sera) (Brugge and Erikson 1977). The heavy chain of the IgG can be phosphorylated by the enzyme, a reaction which allows one to identify the enzyme (Collett and Erikson 1978; Levinson et al. 1978). We have detected pp60^{src} in ASV virus particles (Schmidt-Ruppin strain of subgroup D, SR-D), used them as a source of the isolation of pp60^{src}, and found it associated with the viral envelope which reflects properties of the host cell membrane.

C. Results

The SR-D virus mixed with ^{35}S-methionine-labeled virus was disrupted in the presence of nonionic detergent and processed for isolation of pp60^{src} by chromatography on a DEAE cellulose and phosphocellulose column chromatography similar to previously published procedures (Moelling et al. 1978; Owada et al. 1981). Protein kinase activity was assayed by ^{32}P incorporation into casein, and furthermore ^{35}S-methionine radioactivity of the fractions was determined by acid precipitation (Fig. 1). Fractions of the phosphocellulose column (16–20) exhibited IgG phosphorylating protein kinase activity and contained ^{35}S-methionine-labeled 60K molecules (Fig. 1, bottom). The partial proteolytic cleavage pattern of the 60K band was indistinguishable from its cellular analog (not shown).

The pp60^{src} purified by this procedure was capable of phosphorylating itself in an endogenous reaction (Fig. 2, slots 1 to 4). Depending on the assay conditions it stayed intact as a 60 K molecule or underwent transition to degraded forms such as 50/52K or even 45K. Lower incubation temperatures were more favorable for conservation of the 60K form. The pp60^{src} was released from virus particles by treatment with nonionic detergent (Fig. 2). After a high-speed centrifugation insoluble material was pelleted (p) which contained 60K and 50/52K molecules, whereas the supernatant (s) revealed only the 50/52K form as tested by endogenous phosphorylation. The pelleted material was floated through a sucrose densitiy gradient for isolation of membranes according to a published procedure (Van de Ven et al. 1978). The pp60^{src} was found to be specifically associated with the viral membranes (m) where it remained in its intact 60K form (Bunte et al., J Virol, in press). The undegraded as well as the degraded forms were capable of phosphorylating IgG of TBR serum (Fig. 2).

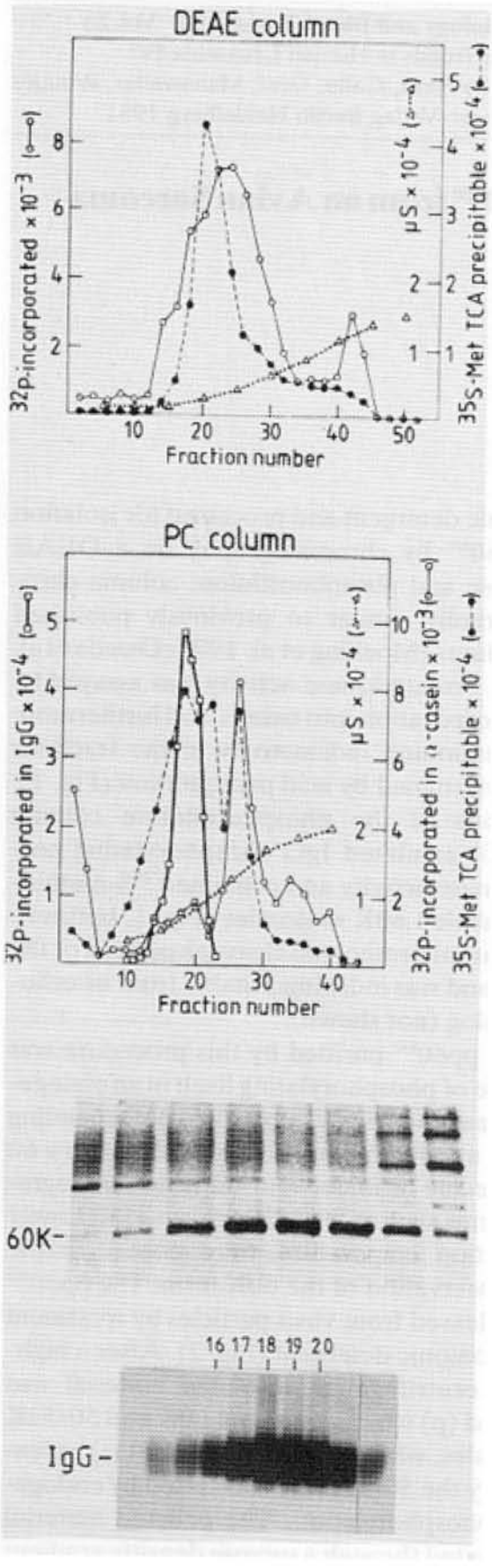

Fig. 1. Purification of ^{35}S-methionine labeled pp60^{src} from SR-D virus particles by DEAE and phosphocellulose column chromatography. Fractions were tested for ^{35}S-methionine radioactivity, presence of pp60^{src} by immunoprecipitation with TBR serum, and IgG phosphorylation according to published procedures (Owada and Moelling 1980)

To furhter analyze the location of pp60^{src} in the viral membrane, intact and disrupted virus particles were analyzed for the accessibility of pp60^{src} to antibodies, surface iodination, and protease. The results are summarized in Table 1. Intact virus did not allow binding of TBR serum as tested for by IgG phosphorylation. Furthermore, no iodination and no endogenous phosphorylation of pp60^{src} or pp50/52K was achieved. Only if disruption of the virus preceded the various treatments did pp60^{src} participate in these reactions. Mild proteolytic digest did not significantly affect pp60^{src}. In parallel, gp85, the viral envelope glycoprotein, was analyzed and gave rise to opposite behaviors. This is in agreement with its position on the outside of the virus and indicates that pp60^{src} is not located there in an analogous fashion.

D. Discussion

From these results a model was deduced (Fig. 3) which shows localization of pp60^{src} inside of the virion. It is embedded in the membrane but not accessible from the outside in contrast to gp85. The 8K moeity of pp60^{src}, which easily breaks off, appears to be hydrophobic, since the 60K molecules can only be kept in solution in the presence of detergent, whereas the 50/52K forms do not exhibit this requirement (Donner et al., unpublished work). The hydrophobic tail may therefore be associated with the lipid bilayer. The pp60^{src} sediments as a globular monomeric molecule (unpublished work) which is schematically indicated by its circular shape.

Whether pp60^{src} is specifically incorporated into the virion from the cellular membrane during the budding process is unknown. Furthermore, it needs to be investigated whether pp60^{src} plays a structural role in the virus particle and whether it is involved in viral transformation.

E. Materials and Methods

Protein kinase assay: 100 μl containing 0.02 *M* MES buffer (N-morpholino-ethane-sulfonic acid) with a pH of 6.8, 10 m*M* $MgCl_2$, 0.625 mg/ml of α-casein, 5 m*M* DTT (dithiothreitol), and 0.1 m*M* [γ-^{32}P]ATP (specific activity 0.5–4 Ci/mmole). Incubation was for 30 min at 30°C. Then acid precipitable radioacti-

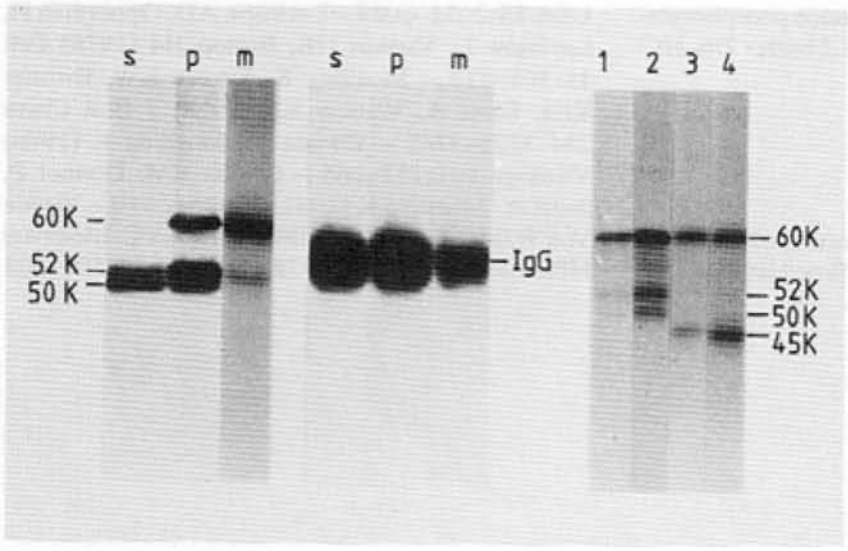

Fig. 2. SR-D virus was disrupted with (2%) NP-40, centrifuged at 45.000 rpm for 180 min at 4°C and separated into supernatant *(s)* and pellet *(p)*. The pellet was used for membrane isolation *(m)* according to a published procedure (Van de Ven et al. 1978). Identical aliquots of s, p and m were incubated for endogenous phosphorylation and IgG phosphorylation (Owada and Moelling 1980). The purified enzyme from the phosphocellulose colum was incubated for endogenous phosphorylation under various conditions to demonstrate its proteolytic cleavage. Incubation was in *1:* 60 min, 0°C, pH 6.8; *2:* 30 min, 20°C, pH 6.8; *3.* 2 h, 0°C, pH 6.8; and *4:* 30 min, 20°C, pH 8.2

Table 1. SR-D without and with disruption (2% NP-40) was used for IgG phosphorylation, for precipitation of ^{3}H-glucosamine radioactivity of the gp85 by antibodies, for surface iodination by means of lactoperoxidase (NEN, Radioch.), and for endogenous phosphorylation. Trypsin treatment (10 μg/ml) was for 30 min at room temperature (nt: not tested)

	Trypsin treatment	IgG-phosphorylation (cpm × 10^3)	H^3-glucosamine gp85 (cpm)	^{125}J-pp60^{src}	^{125}J-gp85 (cpm)	Endogenous phosphorylation		
						^{32}p-60K	^{32}P-50/52K (cpm)	p 19 (%)
Intact	−	52	1360	0	1064	0	0	100
Virus	+	58	603	0	532	0	0	100
Disrupted	−	512	964	169	809	77	610	100
Virus	+	564	537	146	462	72	680	2
Isolated	−	nt	nt	nt	nt	452	41	nt
Membranes	+	nt	nt	nt	nt	39	398	nt

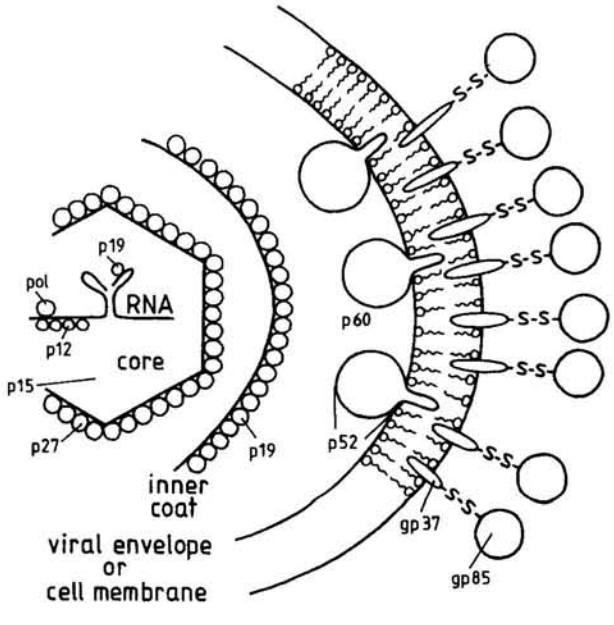

Fig. 3. Schematic presentation of the location of pp60^{src} in an avian RNA tumor virus particle. *gp*, glycoprotein

vity was determined. For endogenous phosphorylation casein was omitted. For pH 8.2 buffer Tris-HCl was used.

References

Brugge JS, Erikson RL (1977) Nature 269:346–348 – Collet MS, Erikson RL (1978) Proc Natl Acad Sci USA 75:2021–2024 – Levinson AD, Opperman H, Levintow L, Varmus HE, Bishop JM (1978) Cell 15:561–572 – Moelling K, Sykora KW, Dittmar KEJ, Scott A, Watson KF (1978) J Biol Chem 254:3738–3742 – Owada M, Moelling K (1980) Virology 101:157–168 – Owada KM, Donner P, Scott A, Moelling K (1981) Virology 110 – Van de Ven WJM, Vermorken AJM, Onnekink C, Bloemers HPJ, Bloemendal HJ (1978) Virology 27:595–603