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Conversion of *ras* Genes to Cancer Genes

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The cellular precursor of Harvey and Balb sarcoma virus, termed proto-*ras*, from certain tumors registers most frequently as an apparent cancer gene in the gene transfer assay with NIH 3T3 cells [1–4]. The viral *ras* gene is a dominant transforming gene that elicits all features of oncogenic transformation in a single step upon transfer into established and primary human and animal fibroblast cells [5]. This encodes a single transforming protein of 21 kDA, p21 Ha-*ras*, which is presumed to be involved in the growth regulation of cells similar to G-proteins [6, 7]. However, the role of the cellular *ras* gene as a human cancer gene is debatable, as only 10% of the DNAs from certain cancer cells produce foci of transformed NIH 3T3 cells in the transfection assay. No consistent correlation between *ras* activation and any special type of human cancer is observed, thus 3T3-transforming proto-*ras* may not be necessary for malignancy [8, 9].

Activation of proto-*ras* in the 3T3 cell assay is due to point mutations in codons 12, 13, 59 or 61 [10–12]. The *ras* coding region in viruses also differs from the coding region of proto-*ras* in normal cells at codon 12 (Balb SV) [13] and codons 12 and 59 (HaSV) [14]. By analogy with the proto-*ras* genes, these point mutations are also thought to activate viral *ras* in sarcoma viruses [15]. To test this hypothesis, parts of viral *ras* including codons 12 (*Hin*dIII-*Pvu*II fragment; proviral DNA clones: pA12, pB12) or 12 and 59 (*Sac*II-*Fsp*I fragment: pA1259-1 or *Hin*dIII-*Fsp*I fragment: pA1259-2) were exchanged in proviral DNA clones against the corresponding fragments of normal rat proto-*ras* 2. One proviral DNA clone derived from pA1259-2 included a *Sac*I-deletion of *ras* sequence directly 5′ of the p21 coding region (pA1259-2ΔSc) (see Figs. 1, 2). Transfection of proviral DNA clones into NIH 3T3 cells (from S. Aaronson, NIH, Bethesda) clearly showed that viral *ras* containing normal codons 12 or 12 and 59 maintain a transforming function (Fig. 3). With clones pA12, pB12, pA1259-2 and pA1259-2ΔSc only a minor reduction of transforming efficiency was observed, whereas pA1259-I showed tenfold less transforming efficiency (Table 1).

Finally, the complete coding region of normal rat p-*ras* 1 (including introns) was inserted into retro viruses to construct pAprasIX and pBpras1. Both proviral clones showed efficient transforming function in NIH 3T3 cells (see Fig. 2 and Table 1). A slight delay of focus formation of 2–3 days already observed with pA1259-1 and pA1259-2 was most pronounced with pAprasIX and pBpras1, probably due to the splicing out of introns during the first rounds of virus replication. Infection of primary Fischer rat embryo fibroblasts with virus derived from pAp*ras*1X DNA demonstrated transforming ability similar to wild type virus (Fig. 3). A few of these viruses will be reisolated and sequenced to verify the identity of the introduced codons of p21.

Inoculation of 10^4–10^5 ffu of each virus derived from the proviral clones described above into 1–5 day old Balb/c mice resulted in death of the animals within 2–8 weeks. Typical signs of malignancy, such as tumor

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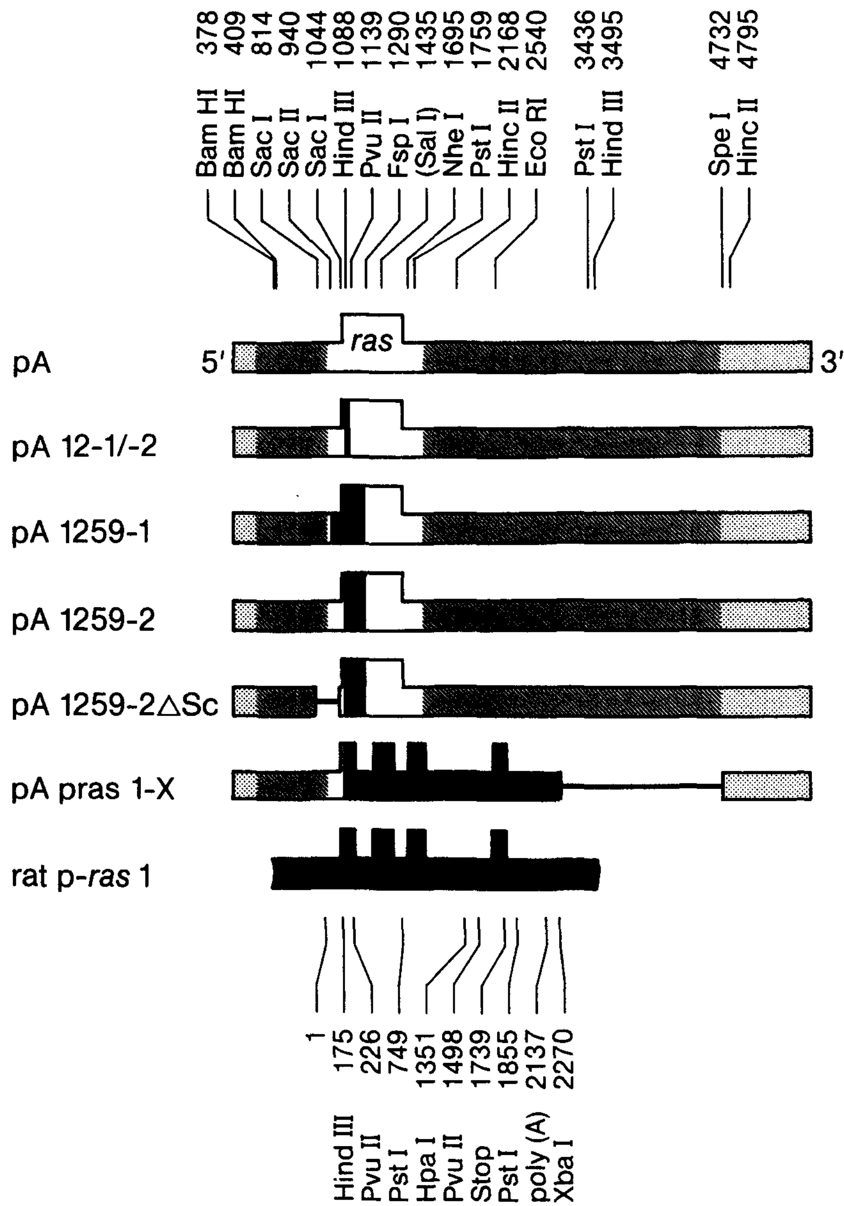
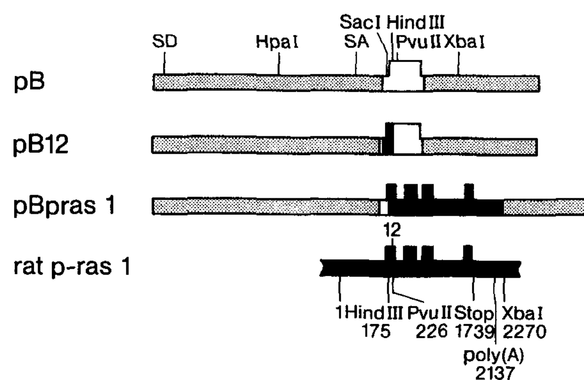


Fig. 1. Genetic structure of proviral DNA of Harvey sarcoma virus, rat proto-*ras* 1 and recombinant virus constructs



	ffu/μg	Latency period
pB	150-300	10 d
pB12	150-250	10 d
pBpras1	10	15 d

Fig. 2. Genetic structure of proviral DNA of Balb sarcoma virus, rat proto-*ras* 1 and recombinant constructs. The table shows the latent period in days for transforming activity after transfection into NIH 3T3 cells in the presence of Moloney MLV

Table 1. Transforming activity of proviral DNA of wild type and recombinant Harvey sarcoma virus after transfection into NIH 3T3 cells

Clone	Ras codon				Foci per µg DNA	Days first seen
	12	59	122	MLV		
pA	v	v	v	+	1000–2000	4
pA	v	v	v	−	40	14
pA12-1/-2	p	v	v	+	1000–2000	4
pA12-1/-2	p	v	v	−	4	14
pA1259-1	p	p	v	+	100– 200	6– 8
pA1259-2	p	p	v	+	300– 400	5– 6
pA1259-2ΔSac	p	p	v	+	1000–1500	4
pApras2	p	p	p	+	0	–
pApras1-X	p	p	p	+	140– 160	10–14

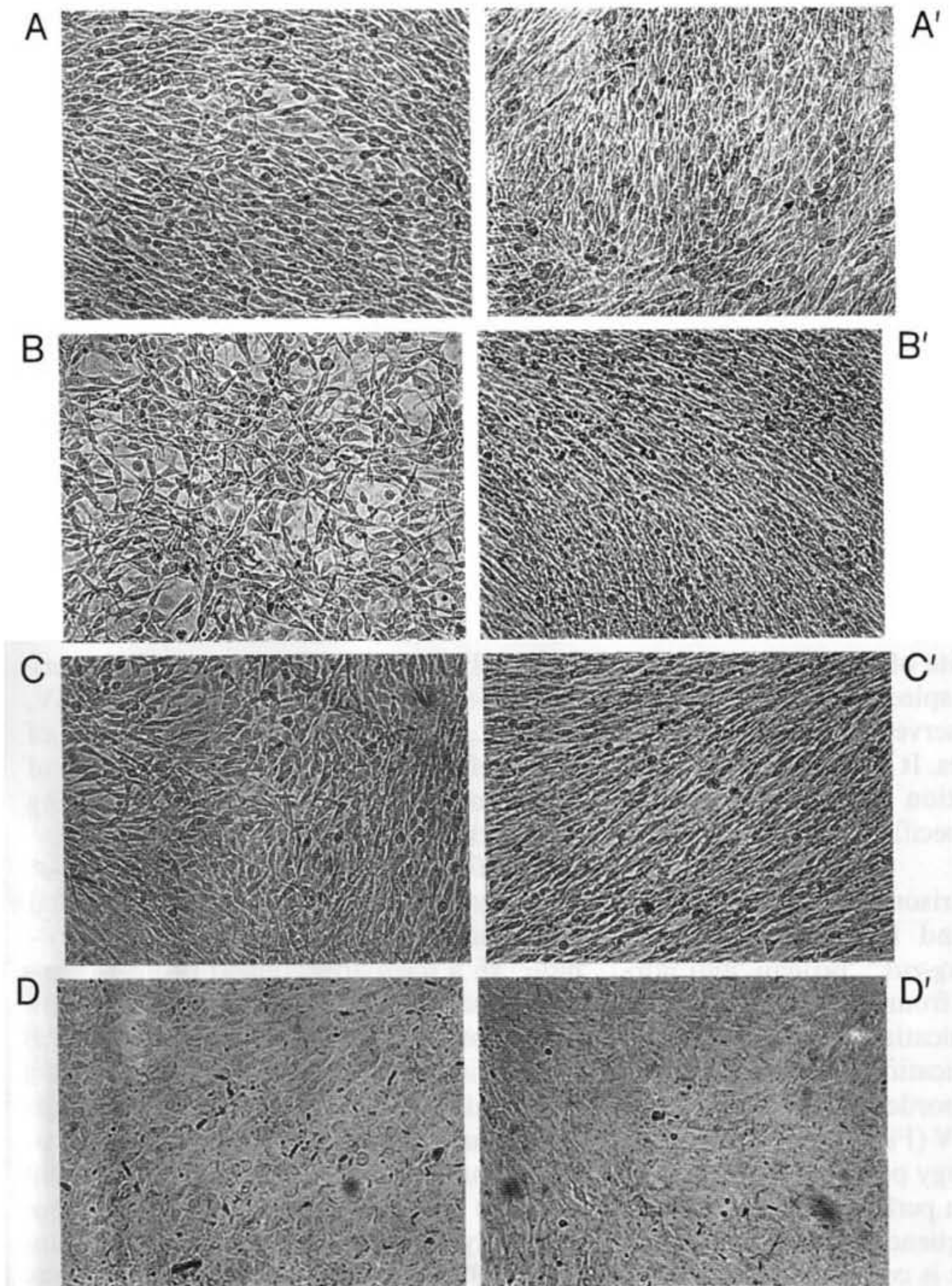


Fig. 3 A–D, A′–D′. Mouse NIH 3T3 cells (**A–D**) and primary Fischer rat cells (**A′–D′**) infected with wild type (**A** and **A′**) and recombinant Harvey sarcoma viruses pA12-2 (**B** and **B′**), pA1259-2 (**C** and **C′**), **D** and **D′** and helper virus (Moloney-MLV)

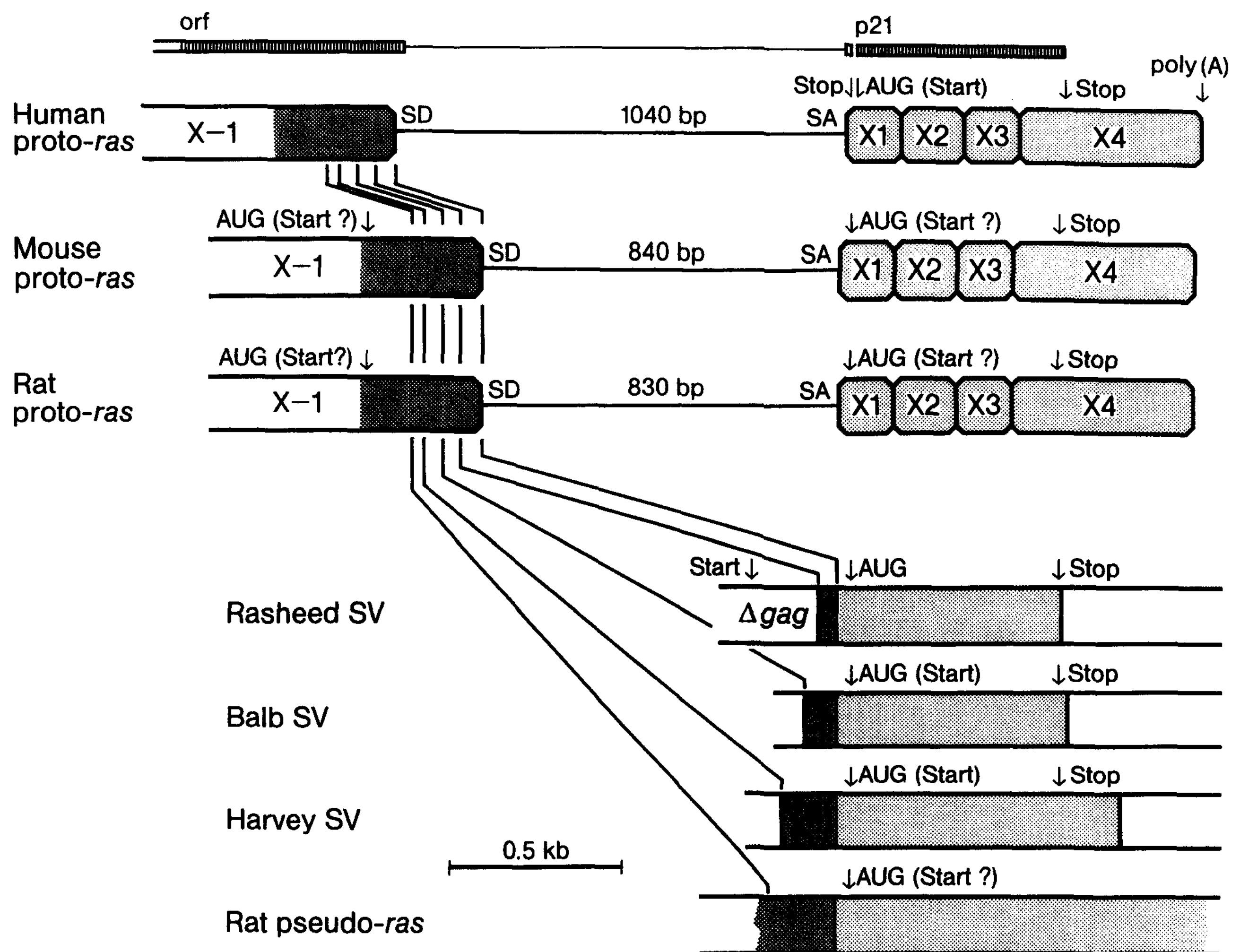


Fig. 4. Sequence comparison between *ras*-containing sarcoma viruses and human, mouse, and rat proto-*ras* genes

formation at the site of injection (sarcoma) or dark nodules in spleen and liver (erythroblastosis), were observed with wild-type and recombinant viruses. It is concluded that the transforming function of viral *ras* is independent of virus-specific point mutations in codons 12 or 59.

Sequence comparison between viral *ras* in HaSV, Balb SV and Rasheed SV, which expresses a p29 gag-ras^{Ha} protein, and normal proto-*ras* 1 from rat (B. Rapoport, personal communication), mouse (J. Ihle, personal communication), and humans revealed a common border of homology at position 1037 of HaSV (Fig. 4). About 1 kb upstream the homology picks up again. At the homology junction perfect splice donor and splice acceptor sequences are present in the proto-*ras* genes. It is concluded that proto-*ras* 1 in humans, rats, and mice contains a previously undetected 5′ exon, termed exon-1, that is only partially present in HaSV, Balb SV, and Rasheed SV. Truncation of exon-1 of p-*ras* 1 and therefore truncation of the normal proto-*onc* gene occurred during transduction into the retroviruses [16].

Therefore we propose that substitution of the native promoter and as yet undefined upstream regulatory regions by the promoter of a retrovirus convert proto-*ras* to a transforming gene. Morphological transformation of 3T3 cells by intact proto-*ras* genes with point mutations may reflect a biochemical effect that enhances tumorigenicity, but does not initiate malignant transformation like viral *ras* genes. It is consistent with this view that 3T3 cells transform spontaneously at high frequency and that untreated 3T3 cells are aneuploid and already tumorigenic [17].

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