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Retroviruses with Two Oncogenes

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A. Introduction

Less than a decade ago the first retroviral oncogene was discovered [1] and shown to derive from a normal cellular gene [2]. To date over 20 different retroviral oncogenes have been found, all derived from cellular counterparts. How a single oncogene can cause cancer is still not fully understood. Natural cancers are known to involve multistep molecular changes, raising a paradox, as compared to cancers induced by retroviral oncogenes. To turn a cellular gene into an active retroviral oncogene, an overexpression due to the very potent retroviral promoter may suffice in a few cases, whereas in most cases several modifications are probably required. These modifications might involve enhanced transcription, truncation of the gene, specific mutations, deletions or insertions (Fig. 1) as well as the viral route of transmission.

In this respect a retroviral oncogene may then be regarded as a single gene having accumulated stepwise, through many replication cycles and selections by researchers (these viruses do not exist in nature) several modifications in order to become a potent cancer gene. Such a process represents a somewhat artificial situation difficult to be challenged for statistical reasons by a cellular oncogene during the lifetime of the host cells. Nevertheless, cellular oncogenes that were identified through retroviruses seem to be involved in some natural cancers, where they are for example deregulated through chromosomal translocation (*c-myc*) or mutations [13] (*c-ras*). Although they appear much "weaker" transforming genes than their viral counterparts, they legitimate the use of retrovirus as tools. It follows that nat-

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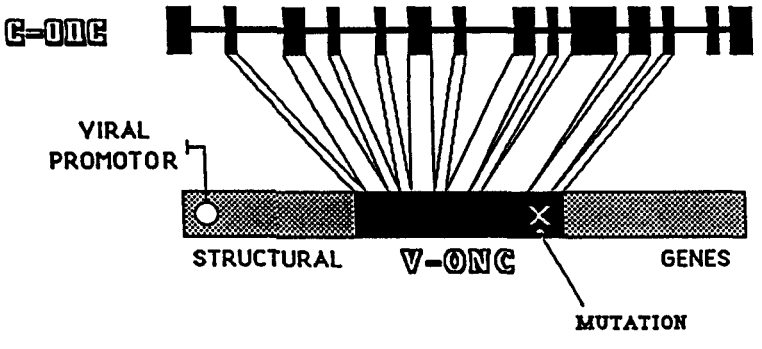

- Transduction of a truncated gene
- Linkage to structural viral genes
- Transcription under a strong viral promotor
- Possible mutations in the transduced gene

Fig. 1. Possible differences between v-*onc* and c-*onc*

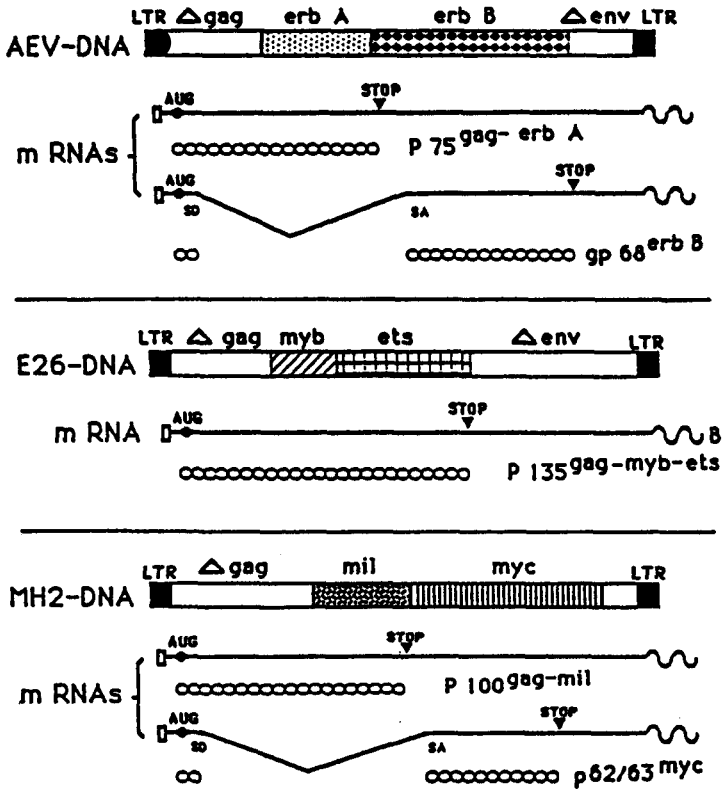

Fig. 2. Retroviruses with two oncogenes

ural cancers are likely to involve single alterations on several distinct genes that cooperate in the tumorigenic process. Recently, a first example of cooperation was demonstrated by in vitro studies showing that the activated *ras* and *myc* oncogenes could transform a rat embryo fibroblast in a way that neither gene could achieve alone [3] and some human tumor cell lines were found to contain the cellular *ras* and *myc* oncogenes both altered. How can we identify more such cooperative genes? Retroviruses may again represent useful tools. In 1979, studying acute avian leukemia retroviruses, we raised the possibility [4] that some retroviruses undergoing modifications could have, among those transduced, not one, but two distinct cellular genes that could cooperate in the viral transformation processes. There are now three examples of such retroviruses (Fig. 2) that we would like to review briefly.

B. Avian Erythroblastosis Virus

The genome of avian erythroblastosis virus (AEV) contains the two oncogenes *erbA* and *erbB* [5]. Deletion mutants in one or the other oncogene have shown that in adult bone marrow the erbB product transforms erythroblasts which subsequently cannot mature properly anymore. The erbA product alone does not modify erythroid cells in a detectable way [6]. In contrast, wild type AEV tightly blocks erythroid cells at an immature stage (erythroid colony forming units or CFUe) [7]. Thus, erbA potentiates the transforming activity of the *erbB* gene product. The nucleotide sequencing of the *erbA* gene appears unrelated to any of the oncogenes characterized so far and related to carbonic anhydrases, enzymes known to play a major role in red blood cells, the progenitors of which are precisely the target cells for AEV. Other studies performed on embryonic tissue of the primitive streak indicate that AEV affects also target cells that appear to precede the BFUe stage.

C. Avian Erythroblastosis Virus E26

The genome of E26 virus contains the two oncogenes *myb* and *ets* [8], expressed in infected cells as a triple fusion protein $P130^{gag\text{-}myb\text{-}ets}$ [5]. E26 appears able to transform uncommitted erythroid-myeloid hemopoietic cells as well as cells committed in the erythroid and myeloid lineages (see Moscovici et al., this volume). How the two oncogenes

myb and *ets* are involved in the transforming properties of E26 virus awaits the construction of mutants deleted in one or the other of the genes. We recently obtained a molecular clone of E26 provirus that is biologically active (D. Leprince et al., in preparation) and that should facilitate the construction of such mutants.

D. Mill-Hill-2 Retrovirus

The avian retrovirus Mill-Hill-2 (MH2) is a replication defective retrovirus that causes, like other avian *myc*-containing retroviruses (MC29, CMII, OK10), mainly liver and kidney carcinomas in the chicken, and transforms chicken fibroblasts and macrophages in culture [5]. MH2 appears more aggressive than the other *myc*-containing viruses in its tumorigenic potential and its genome contains a second oncogene, *mil* [9], yielding in infected cells the two onc proteins $P100^{gag\text{-}mil}$ and $p62/63^{myc}$ [10]. In order to examine the respective roles of the two proteins in the transformation process, we attempted the isolation of spontaneous or constructed mutant expressing properly only the *mil* or only the *myc* oncogene product. Two classes of spontaneous mutants were isolated by Calothy's group (Institut du Radium, Orsay, France) using fibroblasts and neuroretinal (NR) cells prepared from 7-day-old chicken embryos. The choice of this latter cell system was bound to the observation that MH2 wild type virus was shown to stimulate the growth and to transform NR cells that remain usually quiescent in culture ([11]; Table 1). Such an effect was not detected with retroviruses like MC29 (Table 1) that contain only the *myc* oncogene [2] and readily transform chicken fibroblasts [5]. Class I mutants (Table 1) were selected on the basis of their inability to induce NR-cell proliferation, whereas they still transformed fibroblasts. MH2 Cl 16 exhibited such properties and was shown to suffer an extensive deletion in the *mil* gene [12]. A mutant (*MH2-OB*) with similar biological properties was constructed by inducing a frameshift in the *v-mil* gene in a molecularly cloned MH2 provirus, resulting in a premature termination of the v-mil translation product [13].

The class II mutant (i.e., *MH2 PA 200*) was selected on the ground that it induced NR cells to proliferate with the same efficiency as wt-MH2, but failed to transform fibroblasts (Table 1). This mutant also failed to morphologically transform NR cells and showed upon analysis that it was extensively deleted in the *myc* gene [14]. A mutant (*MH2 LI 200*) with similar biological properties (Table 1) was constructed by inducing a frameshift in the *v-myc* gene of molecularly cloned MH2 provirus that resulted in a premature termination of the v-myc translation product.

The results presented here [13] indicate that the ability to induce sustained proliferation and transformation of NR cells from 7-day-old chicken embryos is a remarkable property distinguishing MH2 among other *myc*-containing retroviruses, and requiring the coordinate expression of both *mil* and *myc* oncogenes. Class I mutants lacking a functional *mil* gene (*MH2 Cl 16, MH2 OB*) do not induce NR-cell proliferation nor transformation, although they still transform fibroblasts (and macrophages [15]). Conversely, mutants expressing only the *mil* oncogene (*MH2 PA 200, MH2 LI 200*) induce NR cell proliferation without morphological transformation.

So far, the viral *myc* oncogene (or the large T of polyomavirus) was shown to cooperate with the EJ bladder carcinoma activated *ras* oncogene (or middle T of polyomavirus) for rat embryo fibroblast transformation [3]. We show now that the *myc* oncogene can also cooperate with the *mil* on-

Table 1. Characterization of *MH2* mutants

Virus	Fb1 T	NR		Apparent functional oncogene	
		M	T		
MC29	+	−	−	*myc*	
wt MH2	+	+	+	*myc+mil*	
MH2 Cl 16	+	−	−	*myc*	(Class I)
MH2 OB	+	−	−	*myc*	
MH2 PA 200	−	+	−	*mil*	(Class II)
MH2 LI 200	−	+	−	*mil*	

Fb1, fibroblasts; NR, neuroretinal cells; T, transformed; M, mitogenized; wt, wild type

cogene (the latter being structurally related to the *src*-gene family [16]) for the transformation of NR cells. Thus, the *myc* oncogene may cooperate with two distinct types of oncogenes depending on the cell types considered. Whether *myc* plays a key role in two distinct pathways leading to transformation, or whether the three types of oncogenes (*myc*-, *ras*-, and *mil*-like) belong to a single pathway leading to cell-growth stimulation, where transformation can occur when some of them become deregulated, whereas others might be perhaps constitutively expressed at specific stages of cell maturation or in specific cell types, remains to be examined. Earlier work on polyoma might be recalled in this respect. Although the oncogenes of this virus were not shown to have cellular counterparts, the large *T* and middle *T* polyoma genes were shown to cooperate for fibroblast transformation, as do the activated *myc* and *ras* genes, respectively [17]. In addition, the middle T was shown [18] to bind specifically in the transformed fibroblast the cellular protein $pp60^{src}$ that was shown activated in the complex.

In conclusion, research is slowly beginning to unwrap the cooperation of genes in normal and pathological cell-growth stimulation, and retroviruses with double oncogenes represent convenient tools in such investigations. They have allowed discovery of three new oncogenes (*erbA*, *ets*, and *mil*) that potentiate previously described oncogenes (*erbB*, *myb*, and *myc*, respectively) in their transforming activity or allow these viruses to transform new target cells. Whether the corresponding couples of cellular oncogenes participate in the formation of natural human cancers is under investigation.

References

1. Stéhelin D, Guntaka RV, Varmus HE, Bishop JM (1976) J Mol Biol 101:349–365
2. Stéhelin D, Varmus HE, Bishop JM, Vogt PK (1976) Nature 260:170–173
3. Land H, Parada LF, Weinberg RA (1983) Nature 304:596–602
4. Roussel M et al. (1979) Nature 281:452–455
5. Graf T, Stéhelin D (1982) Biophys Biochim Acta 651:245–271
6. Frykberg L et al. (1983) Cell 32:227–238
7. Samarut J, Gazzolo L (1982) Cell 28:921–929
8. Leprince D et al. (1983) Nature 306:395–397
9. Coll J et al. (1983) Embo J 2:2189–2194
10. Pachl C, Biegalke B, Linial M (1983) J Virol 45:133–139
11. Calothy G et al. (1979) Cold Spring Harbor Symp Quant Biol 44:983–990
12. Martin P et al. (1985) J Virol 57:1191–1194
13. Bechade et al. (1985) Nature 316:559–562
14. Martin P et al. (1986) Virology (in press)
15. Graf T et al. (1986) Cell 45:357–364
16. Galibert F et al. (1984) EMBO J 3:1333–1338
17. Cuzin F (1984) Biophys Biochim Acta 781:193–204
18. Courtneidge SA, Smith AE (1983) Nature 303:435–439