View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Cell, Vol. 86, 691–693, September 6, 1996, Copyright ©1996 by Cell Press

Operating under a Gag Order: a Block against Incoming Virus by the *Fv1* Gene

Minreview

Stephen P. Goff
HHMI and Depts. of Microbiology and Biochemistry
and Molecular Biophysics
Columbia University
New York, New York 10032

The 1996 Cold Spring Harbor Retrovirus Meeting opened with the triumphant and long-awaited report by Dr. Jonathan Stoye of the isolation of the *Fv1* gene of the mouse. This gene, conferring resistance to murine leukemia viruses in the early stages of infection, has a history spanning a quarter-century and has long been considered a Holy Grail of retrovirus biology. The identity of the gene immediately suggests a mechanism for its action, a basis for its evolutionary origin, and a clear program of future work to exploit the finding. Its cloning by the Stoye laboratory at the NIMR in London, now reported in a recent paper in Nature (Best et al., 1996), is particularly gratifying in having been achieved by the lab that has attacked the problem over many years with the most single-minded devotion.

The *Fv1* gene is one of a series of mouse genes originally identified by Lilly in the early 1970s (Lilly and Pincus, 1973; Rowe et al., 1973; comprehensively reviewed by Jolicoeur, 1979). These genes control the susceptibility of mice to leukemia induced by the Friend virus. Many of the genes in the collection (e.g., *Fv2*, *Rfv3*) were found to modify target cell proliferation or the immune response to the virus and not to directly block virus replication; but the *Fv1* gene was cell autonomous, and cell lines derived from Fv1-resistant animals were resistant to virus in culture (Hartley et al., 1970; Pincus et al., 1971). One other gene (*Fv4*) also induced resistance in vitro, but this gene proved to encode an endogenous retrovirus envelope gene (Gardner et al., 1986) and to prevent entry by the well-known mechanism of blocking the virus receptor. By the 1980's, the *Fv1* gene remained alone among the series as a cell-autonomous resistance gene whose mode of action was still unclear.

There are two major naturally occurring *Fv1* alleles among inbred strains: the *Fv1*^{n} allele, found in NIH swiss mice, allowing replication of N-tropic strains of virus and blocking B-tropic strains; and the *Fv1*^{b} allele in Balb/c mice, allowing replication of B-tropic viruses and blocking N-tropics. (A third allele, *Fv1*^{nr}, a variant of *Fv1*^{n}, is present in some strains.) A potentially null allele, later termed *Fv1*^{0}, is present in wild mice that are fully sensitive to all strains of virus (Hartley and Rowe, 1975). Curiously, virus resistance is dominant in genetic crosses, so that *Fv1*$^{n/b}$ heterozygous animals are resistant to both N- and B-tropic viruses. The block to infection mediated by a resistance gene is not absolute, but quite strong, reducing the frequency of infection by two to three logs. Virus infection of resistant cells was found to be blocked at a particularly intriguing step early in the course of infection (see Figure 1): largely after reverse transcription of the RNA genome into linear DNA, but before entry into the nucleus or integration of the DNA into the host genome (e.g., Jolicoeur and Baltimore, 1976). There was some hope that understanding the mechanism of resistance would be revealing about the early phases of the virus life cycle, and might provide a new way to block infection.

The Target of Fv1 Action on the Incoming Virus

Various MuLV isolates could be typed as either N-tropic (able to infect *Fv1*$^{n/n}$ mice), B-tropic (able to infect *Fv1*$^{b/b}$ mice) or NB-tropic (able to infect cells of any genotype). The viral gene that determined the tropism of a virus was eventually identified as the *gag* gene, encoding the precursor of the major virion structural proteins. Mutant viruses with altered tropism could be obtained by forced passage on a nonpermissive host (Hartley et al., 1970; Yoshikura, 1975), and the recovered viruses consistently showed changes in the capsid (CA) domain of the Gag protein. The crucial amino acid sequences were found to lie in a small patch near the center of CA (DesGroseilliers and Jolicoeur, 1983; Ou et al., 1983). Today these observations remain the best evidence that Gag proteins have functions early in infection, and must remain with the viral DNA in a preintegration complex during entry. Presumably the CA protein enters the cell with the virus, stays with the DNA after reverse transcription, and provides a target for the Fv1 gene product to block its nuclear entry and integration. Early speculative models for the dominant action of *Fv1* on the incoming Gag included its role as a sequence-specific protease to degrade the incoming virion, and as a sequence-specific glue that somehow gummed up virus progression.

More data was provided in several labs to expand on the notion of Gag as a target of *Fv1*. Mixed viral particles generated by coinfection of cells with N- and B-tropic viruses are sensitive to restriction by either *Fv1*$^{n/n}$ or *Fv1*$^{b/b}$ mice; the presence of both N-tropic and B-tropic

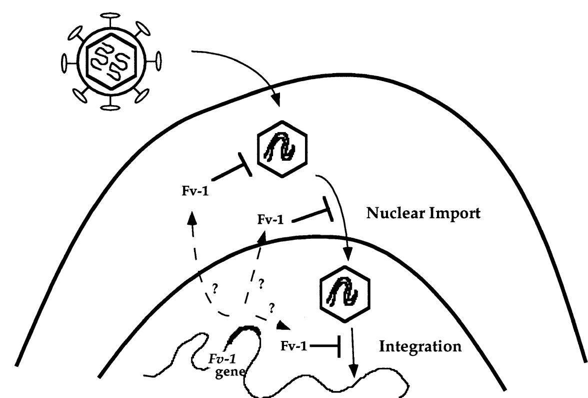

Figure 1. Blocking Incoming Retroviruses inside the Infected Cell by the *Fv1* Gene

The Fv1 gene product, now identified as a Gag-related protein of an endogenous retrovirus-like element, is able to block virus in the early phase of the viral life cycle. The course of infection is blocked after reverse transcription, but before the establishment of the integrated provirus in the host genome. Whether Fv-1 acts only in the cytoplasm, nucleus, or both is uncertain.

Gag proteins on the same virion provides targets for either allele of the *Fv1* gene (Rein et al., 1976; Kashmiri et al., 1977). There was evidence that the incoming Gag was able to saturate the block. When a given virus was titered on a nonpermissive host, the residual infection by that virus did not follow the normal first-order kinetics with dilution, but instead roughly gave second-order (two-hit) kinetics (Decleve et al., 1975; Jolicoeur and Baltimore, 1975; O'Donnell et al., 1976). Further, the block to incoming virus could be transiently abrogated by exposure of the cells to a high concentration of inactivated virions (Duran-Troise et al., 1977). These data were consistent with the notion that a sufficiently large dose of incoming Gag could titer out the Fv1 product and allow the remaining live virus to escape the restriction.

The Plot Thickens: The Identity of Fv1

Stoye's findings now explain how *Fv1* probably works. The gene was isolated by positional cloning. Beginning with a marker nearby on chromosome 4, successively smaller YACs and cosmids were recovered, with the gene being scored by direct bioassay: candidate DNAs were tested after transfection for their ability to render the cells resistant to subsequent challenge with genetically marked virus. Astonishingly, the activity localized to a small, intronless ORF with sequence similarity to the HERV-L family of human endogenous retroviruses (60% identity over 1.3 kb; Cordonnier et al., 1995). Similar elements are widespread in mammalian genomes and are transmitted in trans by helper retroviruses as defective genomes. Based on its position in the element, the *Fv1* gene is apparently a *gag* gene, but a very peculiar one, having only very little similarity to known *gags*. The $Fv1^n$ allele differs from $Fv1^b$ by several point mutations and a different C-terminal sequence, presumably accounting for the specific block against B- and N-tropic Gags. The locus is expressed at excruciatingly low levels. What is appealing about the result is that Gag proteins are known to bind tightly to each other, and to do so via complex interaction domains. The simple model for *Fv1* action is that the HERV-L Gag can bind to the Gag of incoming preintegration complexes and block its normal movement into the nucleus for DNA integration, or its function once there. The *Fv1* version of this HERV-L Gag must be extraordinary one - a dominant negative mutant - since most Gags do not mediate a superinfection barrier.

The identification of *Fv1* as a *gag* has several nice features. First, the result gives us a more concrete picture of the resistance, accounting for the specificity of the alleles for subtly different Gag proteins, the aberrant titration behavior of virus on nonpermissive cells, and the abrogation of resistance by inactivated virions. It accounts for the behavior of mixed particles, which would carry CAs able to bind either the $Fv1^n$ or $Fv1^b$ allelic HERV-L Gag proteins. Second, the finding means that a dominant negative version of a Gag protein that is resident at very low levels in a cell can exert strong resistance against incoming virus. It may turn out that one or a very few Fv1 molecules can inactivate one preintegration complex (containing, at least upon cell entry, about 1500 CA molecules). This may herald further good news in the AIDS field, especially to those hoping to use dominant negative versions of viral genes to similarly render cells resistant to virus. Third, importantly, the result suggests a reason for the mouse to retain such an endogenous retroviral element as a helpful gene during evolution. Mice carrying *Fv1* would show a selective advantage over *Fv1* null littermates in resisting disease; indeed, active *Fv1* alleles are found only in mice carrying many endogenous MuLVs, and not in related species with fewer endogenous proviruses. The finding suggests that the repertoire of defective retroviral DNAs carried in the genome has sometimes served purposes useful to the host. And, finally, the result suggests a clear plan for structural biologists to pursue in the future. The recent progress in the determination of the structure of a retroviral CA (Gitti et al., 1996) will quickly permit tests of various models for Gag–HERV-L interactions. Based on these structures, there are indications that the *Fv1* tropism region of CA may indeed be exposed to the outside of the virion core, and a direct biochemical demonstration of the interaction should be imminent.

The identity of the *Fv1* gene as a *gag* gene is a clear example of irony. The resistance gene that was sought for twenty years was right in front of us all the time: it was a portion of the very virus family being resisted. Indeed, the gene was probably once delivered into the mouse germ line by a replication-competent helper virus. The only sad aspect of the story is that two of the original founders of the field—Wally Rowe and Frank Lilly (who passed away this past year, and to whom Stoye's paper is dedicated)—did not live to see this denouement. As virologists they would have loved it.

Selected Reading

Best, S., Le Tissier, P., Towers, G., and Stoye, J. (1996). Nature *382*, 826–829.

Cordonnier, A., Casella, J.-F., and Heidmann, T. (1995). J. Virol. *69*, 5890–5897.

Decleve, A., Niwa, O., Gelmann, E., and Kaplan, H.S. (1975). Virology *65*, 320–332.

DesGroseillers, L., and Jolicoeur, P. (1983). J. Virol. *48*, 685–696.

Duran-Troise, G., Bassin, R.H., Rein, A., and Gerwin, B.I. (1977). Cell *10*, 479–488.

Gardner, M., Dandekar, S., and Cardiff, R. (1986). Curr. Topics Microbiol. Immunol. *127*, 338–345.

Gitti, R.K., Lee, B.M., Walker, J., Summers, M.F., Yoo, S., and Sundquist, W.I. (1996). Science *273*, 231–235.

Hartley, J.W., and Rowe, W.P. (1975). Virology *65*, 128–134.

Hartley, J.W., Rowe, W.P., and Huebner, R.J. (1970). J. Virol. *5*, 221–225.

Jolicoeur, P. (1979). Curr. Top. Microbiol. Immunol. *86*, 67–122.

Jolicoeur, P., and Baltimore, D. (1975). J. Virol. *16*, 1593–1598.

Jolicoeur, P., and Baltimore, D. (1976). Proc. Natl. Acad. Sci. USA *73*, 2236–2240.

Kashmiri, S.V. S., Rein, A., Bassin, R.H., Gerwin, B.I., and Gisselbrecht, S. (1977). J. Virol. *22*, 626–633.

Lilly, F., and Pincus, T. (1973). Adv. Cancer Res. *17*, 231–277.

O'Donnell, P.V., Deitch, C.J., and Pincus, T. (1976). Virology *73*, 23–35.

Ou, C.-Y., Boone, L.R., Koh, C.K., Tennant, R.W., and Yang, W.K. (1983). J. Virol. *48*, 779–784.

Pincus, T., Hartley, J.W., and Rowe, W.P. (1971). J. Exp. Med. *133*, 1219–1233.

Rein, A., Kashmiri, S.V. S., Bassin, R.H., Gerwin, B.I., and Duran-Troise, G. (1976). Cell *7*, 373–379.

Rowe, W.P., Humphrey, J.B., and Lilly, F. (1973). J. Exp. Med. *137*, 850–853.

Yoshikura, H. (1975). J. Gen. Virol. *29*, 1–9.