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Fusin – a place for HIV-1 and T4 cells to meet

Identifying the coreceptor mediating HIV-1 entry raises new hopes in the treatment of AIDS.

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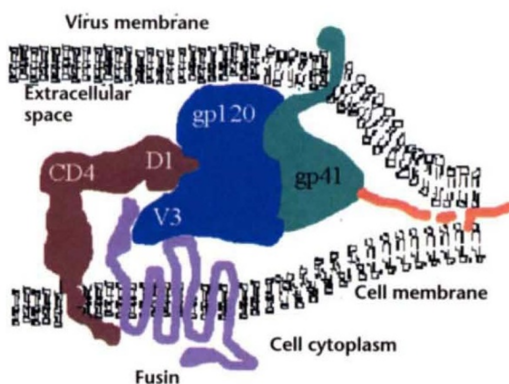
Although it is known that HIV-1 enters human cells via the CD4 molecule, when human CD4 is expressed in mouse cells, HIV-1 is unable to gain entry[1]. This suggests that a human specific cofactor, absent in mouse cells, is required. Despite the efforts of many groups this cofactor has remained elusive. In a recent issue of *Science*, Feng *et al.* report on the identity of a putative cofactor, fusin, which they show is required, in addition to CD4, for HIV-1 entry into human cells[2]. Fusin is a 46 kDa integral membrane glycoprotein and a member of the chemokine receptor family, within the superfamily of G protein-coupled receptors[3]. The identification of this first retrovirus cofactor, tells us that retroviruses can use a variety of membrane-associated molecules, in addition to their primary receptors, to gain access to host cells. It is of critical importance to learn more about these molecules not only to help us understand the "clever games" that viruses play but also to aid in the design of new, highly specific antiviral drugs and methods for gene delivery.

To develop specific fusin-based inhibitors it is helpful to know how fusin interacts with the HIV-1 envelope (the gp120-gp41 complex) and the primary receptor for HIV-1, CD4. One can speculate that because fusin, unlike CD4 (which, by comparison, is an extended, rod-like molecule), has relatively short predicted extracellular portions (a 38 amino acid N-terminus and three even shorter loops), it may help in bringing the fusion peptide of gp41 into close proximity with the host cell plasma membrane (see figure). HIV-1 entry may involve several major steps: (1) the high affinity binding of gp120 to CD4 induces conformational changes in the oligomeric CD4-gp120-gp41 complex resulting in interactions of parts of gp120 (for example, the V3 and V2 loops) with fusin (2) the interaction of fusin with the HIV-1 envelope, and possibly with CD4, helps in the relocation of the fusion peptide from the interior of gp120-gp41 to a position in close proximity to the surface of the host cell membrane (3) the exposed hydrophobic fusion peptide induces local attraction and destabilization of the cell and viral membranes resulting in their fusion. The entry process then continues with formation of a fusion pore and transfer of the viral nucleoprotein complex into the cell interior thus initiating the infection cycle[4].



A sketch of the possible interactions between fusin, viral gp120-gp41 and CD4 resulting in fusion of the viral and host cell membranes. Fusin (purple) may interact with the CD4-gp120-gp41 complex, helping to expose the gp41 fusion peptide (red) which (in concert with other fusion peptides) induces local attraction and destabilization of the cell and viral membranes resulting in their fusion.

What makes these steps in the fusion process an attractive target for intervention is their relatively easy access for a variety of small and large molecules which do not have to cross the cell membrane. The fusion complex is the target for the inhibitory activity of the soluble form of CD4 (sCD4), CD4 immunoadhesins[5] and the gp41 peptide, DP178 (ref. 6). Studies on the inhibition of HIV-1 entry by these molecules provide important pointers for designing drugs targeted against fusin. Such drugs might include molecules which mimic fusin (possibly designed as chimeras with sCD4, DP178 and/or antibodies against gp120-gp41molecules) or which specifically bind to sites where fusin interacts with the CD4-gp120-gp41 complex.

New drugs targeting fusin may help in solving the problem of drug resistance, one of the major limitations of current antiviral drug combination therapies. The virus may well mutate in response to fusin-based drugs but the probability of it finding a substitute cofactor is low. The discovery of fusin also raises hopes for vaccine development. The fusin-CD4-gp120-gp41 complex could be used as an antigen to induce protective immunity. Another alternative is passive immunotherapy using anti-fusin antibodies which should protect against host cell invasion by a number of viral isolates. Indeed, Feng *et al.*[2] show that antibodies to the NH_2-terminal peptide of fusin inhibit HIV-1 envelope-mediated cell fusion and infection. Human anti-fusin antibodies may prove useful in blocking viral entry but with the caveat that they may also interfere with the normal physiological function of fusin, which is presently unknown. However, even for the primary receptor, CD4, which has an important physiological role, an antibody to the second domain of CD4 synergistically inhibited HIV-1 envelope-mediated cell fusion and infection (in combination with anti-gp120 antibodies)[7] but did not induce significant cell loss or immunosuppression in rhesus monkeys[8].

The history of AIDS research is marked with astonishing discoveries followed by great hopes which are often dashed, and teaches us to be cautious when seeking therapeutic applications of new findings. Fusin may prove to be no exception. There are several potential problems in developing fusin-based drugs and vaccines. Unlike the most successful story to date in AIDS treatment, the protease inhibitors (which were designed based on the 3D structure of the viral protease), the structure of fusin is not only unknown but may not be solved in the near future because it is an integral membrane protein. Also, as with other seven-transmembrane proteins, the structure of the whole fusin molecule may have to be revealed in order to understand its function. In spite of the blocking activity of

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antibodies to the N-terminus, fusin peptides may not have the appropriate conformations to exert antiviral effects. This may prevent rational structure-based drug design. More traditional approaches, such as drug screening, may still prove useful. The sites of fusin interactions with the viral gp120-gp41 complex and possibly CD4 are also unknown but their identification is within the capabilities of current methods.

Feng and colleagues show that fusin mediates the entry of viral isolates that prefer to infect T cell lines but not those that favor macrophages as the host cell. Therefore, fusin-based therapy may not prevent infection by all HIV-1 isolates. This problem may soon be resolved when the fusion cofactor(s) for macrophage tropic isolates are identified.

Despite these potential problems, the near future may witness a novel class of drugs based on fusin. The discovery of fusin has solved a puzzle but does not end the story. Instead it has opened an entirely new field of research into virus tropism and entry. Only further investigation will tell us whether this remarkable finding will be translated into prevention and treatment for AIDS and other diseases.

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Ribozyme trans-splicing and RNA tagging: Following the messenger

Splicing ribozymes can be used to repair mutant RNA transcripts in mammalian cells (pages 643–648).

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RNA molecules with enzymatic activity, or ribozymes as they are known, have great potential as therapeutic entities[1,2] due to their ability to either cleave deleterious RNA transcripts[3,5] or repair mutant cellular RNAs[6]. Several ribozyme classes, or catalytic motifs, have been identified, each mediating a naturally occurring biological process. The group I intron of *Tetrahymena* catalyses cleavage and ligation reactions during RNA splicing; RNAse P is required for trimming the 5' terminus of tRNA precursor molecules; simpler ribozymes ('hammerhead' and 'hairpin') are RNAs that catalyse site specific scission of single stranded target RNAs bearing a consensus cleavage site. An example of a ribozyme with a repair function is the *Tetrahymena* group I intron which, during self-splicing, cleaves the phosphodiester bond that attaches the intron to the 5' exon and then ligates its 3' exon to the released 5' exon[7]. The Tetrahymena group I ribozyme (L21 version) can also transfer its 3' exon to a 5' exon of a heterologous transcript in the trans orientation[8,9], a reaction mediated via pairing of the 5' exon to be spliced to a complementary internal guide sequence (IGS; see figure).

In a provocative paper in this issue of *Nature Medicine*, Jones *et al.*[10] show that it is possible to use a splicing ribozyme to 'edit' genetic information encoded by mRNAs inside mammalian cells. Their study uses two model systems to demonstrate this in practice. One system consists of a dual expression plasmid with a T7 promoter driving expression of a 3'-truncated lacZ transcript in one direction and the trans-splicing ribozyme, with the 3' lacZ sequence as the tag, in the other direction. Following transfection of the plasmid into a mammalian cell, the authors demonstrated transfer of the 3' lacZ sequence onto the truncated transcript using PCR amplification and sequence analysis of the repaired lacZ RNA transcript. In an earlier paper[6], this group showed that restoration of lacZ enzymatic activity accompanied transcript repair in a reaction carried out in bacterial cells. In the second system, Jones *et al.* use a retrovirus vector with the SV40 promoter driving ribozyme expression. In this case, the authors show that trans-splicing of the lacZ 3' tag occurs in endogenous nuclear transcripts containing the sequence complementary to the IGS sequence.

There are several implications to this finding. First, one can potentially repair mutant RNA transcripts associated with a variety of genetic diseases such as sickle cell anemia and cystic fibrosis. One of the advantages of this repair approach over conventional gene replacement therapy is that the repaired gene remains within its natural genetic environment without perturbation, a critical factor for regulated gene expression. Such regulated expression has been extremely difficult, if not impossible to achieve using the traditional gene 'add back' approach. A second advantage is the possible treatment of genetic diseases associated with dominant or deleterious mutant proteins where elimination of the mutant transcript is required for clinical benefit (for example, sickle cell anemia or malignancies mediated by mutant oncogenes). By its very nature, RNA repair accomplishes both tasks simultaneously: repair and retention of regulated gene expression. For an acquired disease such as HIV-1 infection, trans-splicing ribozymes can be used to rewrite HIV genetic information (introduce termination codons or other genetic modifications) thereby mediating an antiviral effect. Trans-splicing and RNA repair thus represent a fundamentally different approach to gene replacement therapy as currently practiced.

Another major contribution by Jones