cellular L-arginine concentration (*5, 20*). The concentration of L-arginine required for maximal uptake in endothelial cells is within the physiological range of L-arginine plasma levels (7) [apparent half-maximal saturation constant (K_t) = 0.21 mM; saturation after 15 s]. In contrast, L-arginine uptake in islets is saturated at 15 mM (*21*), a concentration that is about 100 times higher than the normal concentration in plasma. Thus, changes in plasma L-arginine due to diet or other factors would be expected to be a regulator of the oxidative L-arginine pathway in pancreatic B cells but not in endothelial cells.

Exocrine and endocrine stimulus-secretion coupling may represent a physiological function for signal transduction by NO (*22*). The regulation of insulin release by L-arginine has been reported to be deficient in patients with noninsulin-dependent diabetes mellitus (*23*) but not with insulin-dependent diabetes mellitus (*24*). Thus, alterations of the oxidative L-arginine pathway may participate as one of the mechanisms of pathogenesis in diabetes mellitus. Diabetes mellitus may represent only one expression of a putative defect in the oxidative L-arginine pathway. Indeed, diabetes mellitus has been associated with diminution in endothelium-dependent relaxation (*25*) and nonadrenergic, noncholinergic neurotransmission (*26*); both of these processes are known to be mediated by the oxidative L-arginine pathway. These clinical conditions may be explained by altered release of NO or the expression and regulation of different isoforms of NO synthases (*12, 22, 27*).

Note added in proof: L-Arginine–induced increases in cGMP, evidence for a constitutive NO synthase, and inhibition of insulin release by Me Arg in islets of Langerhans from rat and RINm5F insulinoma cells have been reported (*29*).

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Reversal of Integration and DNA Splicing Mediated by Integrase of Human Immunodeficiency Virus

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In retroviral integration, the viral integration protein (integrase) mediates a concerted DNA cleavage-ligation reaction in which the target DNA is cleaved and the resulting 5′ ends of target DNA are joined to the 3′ ends of viral DNA. Through an oligonucleotide substrate that mimics the recombination intermediate formed by this initial cleavage-ligation reaction, the purified integrase of human immunodeficiency virus was shown to promote the same reaction in reverse, a process called disintegration. Analysis of a set of structurally related substrates showed that integrase could promote a range of DNA cleavage-ligation reactions. When the viral DNA component of the disintegration substrate was single-stranded, integrase could mediate a DNA splicing reaction analogous to RNA splicing.

INTEGRATION OF VIRAL DNA INTO THE genome of a new host cell is a critical step in the life cycle of retroviruses. Genetic and biochemical studies of several retroviruses, including human immunodeficiency virus (HIV-1), have shown that integration requires sequences at the ends of the linear viral DNA, and a protein encoded by the viral *pol* gene, the integration protein or integrase (*1, 2*). The viral DNA precursor for the integration reaction is a linear double-stranded molecule that is synthesized by reverse transcription from the viral RNA genome. Two bases from each 3′ end of the linear viral DNA are first removed by integrase such that the viral 3′ ends are recessed by two bases from the 5′ ends and terminate with the dinucleotide CA (*3–8*). A staggered cut is then made in the target DNA, and the resulting overhanging 5′-P ends are covalently joined to the recessed 3′-OH ends of the viral DNA (*3–5, 9*). Target DNA cleavage appears to be coupled to the joining of viral and target DNA because this step does not require an exogenous energy source (*10, 11*). This cleavage-ligation reaction produces a gapped intermediate; integration is completed by a gap repair process that remains to be characterized.

Recent in vitro studies with synthetic oligonucleotides have shown that the processing of the viral DNA ends and strand transfer can be accomplished by integrase alone (*4, 8*). As the cleavage-ligation reaction mediated by integrase is likely to be isoenergetic, we hypothesized that this step would be reversible. In this study, we an-

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nealed synthetic oligonucleotides to produce a structure we term a Y-oligomer (Fig. 1B) (*12*). This structure mimics the product of an integrase-mediated reaction that joins a 21-bp viral DNA right end (U5) to a unique site in a 30-bp target DNA. If the integrase-mediated DNA joining step is reversible, strand cleavage should occur precisely at the junction between the viral and target DNA sequences and should be coupled to the rejoining of the interrupted target DNA sequences. Two products are thus expected as follows: a 19-bp viral DNA fragment containing a two-base overhanging 5′ end and a 30-bp target DNA fragment (Fig. 2A). In a complete reaction (*13*) containing integrase and a Y-oligomer labeled with ^{32}P at the 5′ end of T1, we observed the production of the expected labeled 30-bp fragment (Fig. 2, B and C). When the 5′ end of the hybrid V1/T2 strand was labeled, incubation of the Y-oligomer with integrase released a labeled 19-nucleotide fragment, corresponding in length to the viral component of the hybrid strand (Fig. 2D). The reaction required both integrase and Mn^{2+}. The same reaction was also observed with a Y-oligomer that contained a viral left (U3) end (*14*). We confirmed the identity of the 30-nucleotide product in Fig. 2C by determining its DNA sequence (Fig. 3). We conclude that HIV-1 integrase can precisely reverse the initial DNA cleavage-ligation in retroviral integration. We refer to this reaction as disintegration.

The experiments shown used integrase purified from *Escherichia coli* expressor cells, but similar results were obtained with viral particles prepared from HIV-1–infected cells or HIV-1 integrase purified from yeast expressor cells (*14, 15*). When the same substrate was used, formation of the 30-nucleotide product was not detected when integrase was replaced by any of several other DNA breakage and joining enzymes: Gin recombinase of phage Mu, phage λ Int protein, *E. coli* DNA gyrase, wheat germ topoisomerase I, DNA ligases of *E. coli*, and phage T4 (*14, 15*). The described activity on the Y-oligomer therefore appears to be specific for the HIV-1 integrase. We do not believe, however, that disintegration is an activity unique to HIV-1 integrase. Many key mechanistic features of HIV integration are shared with other retroviruses, retrotransposons, and some eukaryotic and prokaryotic transposons. Indeed, there is evidence that integrase of murine leukemia virus also mediates disintegration (*16*).

By analyzing integrase's action on a set of structurally related Y-oligomer substrates (Fig. 4), we found that the protein has an unexpected ability to carry out diverse template-guided DNA transesterification reactions, in which one DNA 3′-OH substituent at a phosphodiester bond is exchanged for another. Removal of the two unpaired 5′ bases from the V2 strand of the Y-oligomer (Fig. 4A, a) did not adversely affect integrase-mediated disintegration (Fig. 4B). The Y-oligomer with the entire V2 strand omitted (Fig. 4A, b) still retained 10% of wild-type activity (Fig. 4B). Reducing the length of this single-stranded 5′ tail had

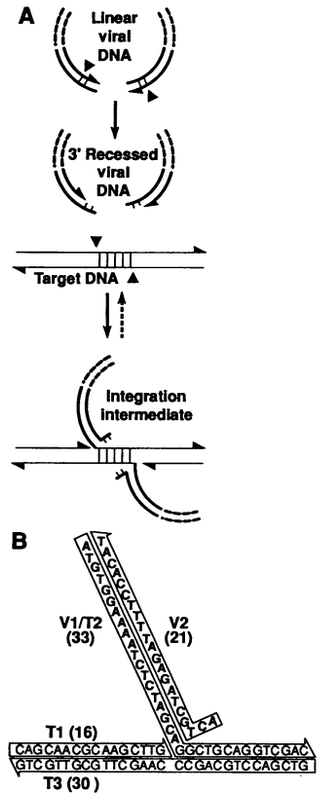

Fig. 1. (**A**) Formation of recombination intermediate. The initially blunt-ended linear viral DNA is cleaved by integrase, resulting in 3′ ends recessed by 2 bases. The target DNA is cleaved with a 5-bp stagger, and the resulting 5′-P ends are joined to the 3′-OH ends of the viral DNA. We shall refer to the DNA joining reaction that gives rise to this recombination intermediate as integration (signified by a solid arrow) and to the reverse reaction that resolves its viral and target components as disintegration (signified by a broken arrow). Arrowheads indicate sites of cleavage or strand exchange. The 3′-OH ends of DNA strands are denoted by half-arrows. (**B**) DNA sequence and structure of Y-oligomer. The Y-oligomer substrate, which resembles the initial recombination intermediate shown in (A), was formed by annealing the following four oligonucleotides: T1, 16-mer; T3, 30-mer; V2, 21-mer; and the hybrid strand, V1/T2, 33-mer. Bold letters are the DNA sequences of the U5 end of the HIV-1 (HXB2) long terminal repeat. Plain letters are target DNA sequences derived from plasmid πAN13 (*21*).

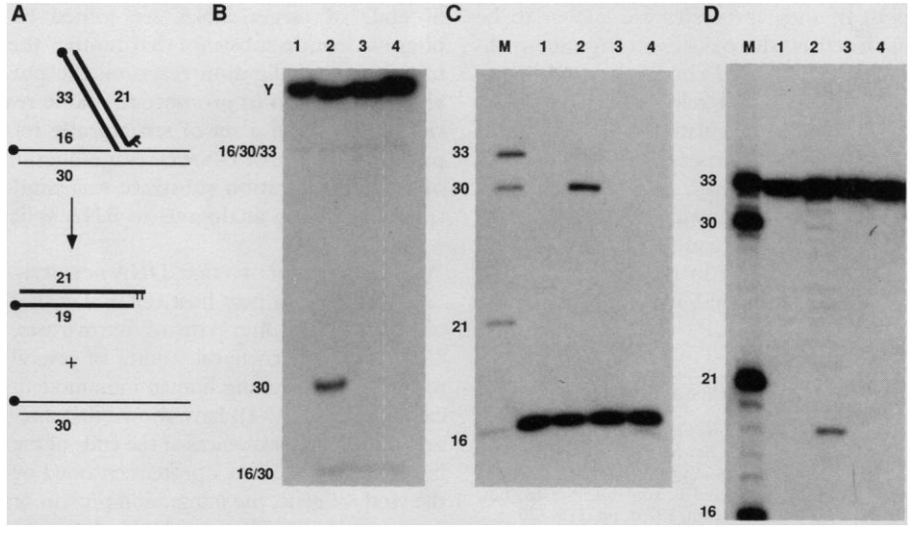

Fig. 2. Strand breakage and joining mediated by HIV-1 integrase. (**A**) Schematic illustration of the expected products after disintegration of the Y-oligomer. Thick lines represent viral DNA sequences, and thin lines represent target DNA sequences. Closed circles denote the ^{32}P-labeled 5′ ends. In (B) and (C), the Y-oligomer was singly labeled at the 5′ end of T1 (16-mer), whereas in (D) the Y-oligomer was singly labeled at the 5′ end of the hybrid strand, V1/T2 (33-mer). The length in nucleotides of each strand is indicated. (**B**) Strand joining product analyzed on a native polyacrylamide gel. Reactions were carried out as described (*13*). The minor bands labeled as 16/30 and 16/30/33 are partially dissociated Y-oligomers: 16/30, labeled T1 annealed with T3; 16/30/33, labeled T1 annealed with T3 and V1/T2. (**C**) Strand joining product analyzed on a denaturing polyacrylamide gel. Reactions were the same as in (B). The smear above the 30-nucleotide band was caused by residual secondary structure present in the labeled product. (**D**) Released viral 3′ end product analyzed on a denaturing polyacrylamide gel. In (B) to (D), lane 1 is the labeled, untreated Y-oligomer substrate; lane 2 is a complete reaction containing integrase and $MnCl_2$; lane 3, integrase was omitted; lane 4, $MnCl_2$ was omitted; lane M contained DNA size markers. The numbers on the left denote the length of DNA in base pairs or nucleotides. The products of length longer than 19 bases in lane 2 of (D) presumably represent reintegration of the released viral end oligonucleotide at new target sites.

Fig. 3. Disintegration restores the continuity of the interrupted target DNA strand. Y-oligomer labeled at the 5′ end of T1 was incubated with integrase, and the reaction products were separated by electrophoresis in a 15% denaturing polyacrylamide gel. The 30-nucleotide strand was excised and extracted from the gel, and the sequence was determined (*22*). The 3′-OH ends of the strands are denoted by half-arrows. Bold letters represent viral DNA sequences, and plain letters represent target DNA sequences. The sequence of the material migrating as a smear above the 30-nucleotide fragment (Fig. 2C, lane 2) was also determined and was identical to that of the 30-nucleotide strand (*14*).

little or no additional effect on activity (Fig. 4, A and B; b, e, and f). Replacing the highly conserved CA dinucleotide with TC (Fig. 4A, c) resulted in a sixfold reduction in disintegration (Fig. 4B). The effect of changing base composition and Y-oligomer structure appeared to be multiplicative: substitution of the CA dinucleotide with TC and omission of the V2 strand (Fig. 4A, d) together decreased the activity almost 65-fold (Fig. 4B).

These results indicate that both the viral DNA sequence and the gross structure of the recombination intermediate influence the disintegration reaction mediated by HIV-1 integrase. A substrate whose sequence and structure most closely mimic the integration intermediate is preferred for disintegration, yet remarkable deviations from this structure are tolerated.

The preference for the authentic joining intermediate as a reaction substrate supports the idea that disintegration is indeed the exact reversal of integration rather than an aberrant DNA breakage and joining activity. It has been proposed that integrase might participate as a reactant in the integration reaction by undergoing an irreversible conformational change. Our demonstration that integrase can catalyze this reaction in both directions is inconsistent with such a model and implies that integrase is not a reactant but an enzyme.

We use the term "DNA splicing" for the DNA cleavage and joining mediated by integrase on partially single-stranded Y-oligomers (Fig. 4A, b, d, e, and f), in recognition of its similarity to excision of introns by RNA splicing (*17*). Like the retroviral integrase, the *Tetrahymena* ribosomal RNA group I intron has been shown in vitro to mediate both splicing and integration reactions, acting, however, on RNA rather than a DNA substrate (*18*). In the most striking example of DNA splicing, we found that integrase could reseal a nick in DNA of arbitrary sequence, provided that there was at least one extra nucleotide at the 5′ side of the nick (Fig. 4A, f). Unlike DNA ligases, integrase could not seal a simple DNA nick (*14*).

The DNA splicing activity of integrase could play a role in joining the viral 5′ end to target DNA, an essential and heretofore unexamined final step in retroviral DNA integration. We speculate that after the single-stranded gap flanking the integrated viral DNA end is filled in, perhaps by reverse transcriptase, removal of the two unpaired bases at the viral 5′ ends and joining of the viral and target DNA could be mediated by the DNA splicing activity of integrase. The DNA splicing activity could also be involved in retroviral recombination. In the strand displacement-assimilation model (*19*), crossing over between the two retroviral genomes during plus strand DNA synthesis could produce an intermediate with a local structure identical to that of the partially single-stranded Y-oligomer (Fig. 4A). Our results raise the possibility that resolution of such an intermediate could be mediated by integrase to produce a heteroduplex molecule and liberate a new 3′-OH end.

The requirements and optimal conditions for disintegration are similar to those for integration (*4, 11, 20*). This suggests that, at least in vitro, both the formation and dissociation of the initial recombination intermediate can take place simultaneously. The poor yield of the integration reaction observed with purified integrase in vitro may therefore be due in part to the counteracting effect of disintegration. However, whether disintegration plays any role in the overall integration process in vivo is presently not known. The net direction of the reaction is likely to depend on physical constraints on the viral and target DNA molecules and the availability of other accessory viral or host factors.

Its biological significance notwithstanding, the disintegration activity provides new approaches for studying integrase and the integration process. The disintegration activity can be used as an additional functional assay for integrase or related enzymes. The disintegration substrate has the advantage that the site of integration into target DNA is predetermined and can be manipulated. This is in contrast to the forward reaction in which the site of integration is not unique (*1, 11*). The disintegration substrate is therefore particularly well suited for studies that benefit from a defined site of integration, such as stereochemical or topological studies of the strand transfer step, and investigations of protein-target DNA interactions during retroviral DNA integration.

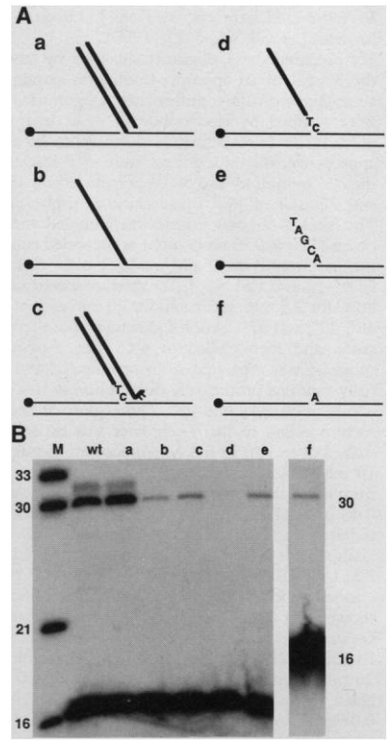

Fig. 4. Effect of nucleotide sequence and structure on integrase-mediated disintegration. (**A**) Sequences and structures of substrates. Closed circles denote ^{32}P-labeled 5′ ends of the strands. Except substrates e and f, letters indicate bases that differ from the wild-type U5 end sequence (Fig. 1B). (**B**) Activity. Reaction products were analyzed in a 15% denaturing polyacrylamide gel. Equal amounts of radioactivity, as determined by Cherenkov counting, were loaded onto each lane. The substrates used in different reactions are indicated at the top of each lane; wt, wild-type U5 Y-oligomer (Fig. 1B); lane M, size markers with the length in nucleotides indicated on the left.

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12. We prepared the Y-oligomer substrate by labeling the 5′ end of an appropriate oligonucleotide and annealing with its complements. Oligonucleotides were purified by electrophoresis in a denaturing polyacrylamide gel before use. Fifty picomoles of the appropriate oligonucleotide were ^{32}P-labeled at their 5′ termini by use of T4 polynucleotide kinase and 25 μCi of [γ-^{32}P]adenosine 5′-triphosphate. The labeled oligonucleotide was annealed with an excess (150 to 250 pmol each) of unlabeled complementary strands in 10 mM tris-HCl, pH 7.5, 1 mM EDTA, and 0.1 M NaCl. The mixture was heated to 80°C for 2.5 min, incubated for 20 min each at 50°, 45°, 40°, and 37°C, cooled slowly to room temperature, and then chilled to 4°C. The Y-oligomer substrate was separated from unannealed and partially annealed products by electrophoresis in a 15% native polyacrylamide gel. The region of the gel corresponding to the Y-oligomer was excised and soaked overnight in 0.5 M ammonium acetate and 10 mM magnesium acetate. The supernatant fluid from the soaked gel fragment was filtered through a 0.45-μm cellulose acetate membrane, then concentrated in a Centricon-10 column (Amicon), and washed with 20 × volume of 10 mM tris-HCl, pH 7.5, 1 mM EDTA, and 0.1 M NaCl. Analysis of the purified labeled substrate by gel electrophoresis showed that more than 95% of the radioactivity was associated with the Y-oligomer.
13. Reactions (20 μl) contained a final concentration of 20 mM Hepes, pH 7.5, 10 mM $MnCl_2$, 20 mM NaCl, 1 mM dithiothreitol, 0.1 mM CHAPS, 0.05% Nonidet P40, 0.1 pmol of Y-oligomer substrate, and 2.8 pmol of HIV-1 integrase. The mixture was incubated for 60 min at 37°C. The reaction was stopped by addition of 18 mM EDTA, pH 8.0, proteinase K (300 μg/ml), and 0.1% SDS, and then incubated for an additional 30 min at 37°C. HIV-1 integrase was purified from *E. coli* expressor cells by a procedure modified from that of Sherman and Fyfe (*8*). The construction of the HIV-1 integrase expression plasmid and purification of integrase will be described elsewhere (*20*).
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15. HIV-1 integrase purified from yeast expressor cells was a gift from A. D. Leavitt and H. E. Varmus, University of California, San Francisco. Viral particles prepared from HIV-1–infected cells were provided by Frederick Cancer Research Facility, National Cancer Institute. *Escherichia coli* DNA gyrase was provided by A. Kornberg, Stanford University; IHF, Fis protein, phage λ Int protein, and Gin protein of phage Mu were provided by N. R. Cozzarelli, University of California, Berkeley.
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Potentially Amyloidogenic, Carboxyl-Terminal Derivatives of the Amyloid Protein Precursor

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The 39– to 43–amino acid amyloid β protein (βAP), which is deposited as amyloid in Alzheimer's disease, is encoded as an internal peptide that begins 99 residues from the carboxyl terminus of a 695– to 770–amino acid glycoprotein referred to as the amyloid β protein precursor (βAPP). To clarify the processing that produces amyloid, carboxyl-terminal derivatives of the βAPP were analyzed. This analysis showed that the βAPP is normally processed into a complex set of 8- to 12-kilodalton carboxyl-terminal derivatives. The two largest derivatives in human brain have the entire βAP at or near their amino terminus and are likely to be intermediates in the pathway leading to amyloid deposition.

IN HUMAN CEREBROSPINAL FLUID AND brain, and in medium conditioned by cultured cells, there are large, secreted βAPP derivatives (*1–4*). These secreted NH_2-terminal derivatives are produced by a cleavage within the βAP (*5, 6*) that also produces small cell-associated COOH-terminal fragments. Because they contain only part of the βAP, the derivatives produced by this cleavage cannot produce amyloid.

To determine if any βAP-bearing, COOH-terminal derivatives are present in human cerebral cortex, we prepared membrane-associated proteins from cortex and passed them over an affinity column made with antibody to $βAPP_{672-695}$ (anti-C_{24}) (*7*). The affinity-purified proteins were then separated by tris-tricine SDS–polyacrylamide gel electrophoresis (PAGE) (*8*), a system that clearly resolves small proteins. The anti-C_{24} column retained full-length forms (Fig. 1A, bracket) and five putative COOH-terminal derivatives (~11.8, ~11.4, ~10.9, ~9.6, and ~8.7 kD) (Fig. 1A, arrows) that were (i) specifically labeled by anti-C_{24} (Fig. 1B, lanes 1 and 2) and (ii) readily detected in brains from patients with Alzheimer's disease (AD) (Fig. 1A) and in control brains (Fig. 1C). As shown in Fig. 1F (lanes 6 and 7), an antibody to $βAPP_{649-664}$ specifically immunoprecipitated the same five 8- to 12-kD proteins immunoprecipitated by antibody to $βAPP_{676-695}$ (anti-C_{20}) (lane 5) or anti-C_{24} (Fig. 1, A and B). The specific recognition of these proteins by antisera against two nonoverlapping epitopes in the COOH-terminal region of the βAPP ($βAPP_{649-664}$ and $βAPP_{676-695}$) provides strong evidence that they are all COOH-terminal βAPP derivatives. When separated by conventional tris-glycine SDS-PAGE, the five 8- to 12-kD proteins migrated above the 14-kD marker as a doublet of ~15 and ~17 kD (Fig. 1D) similar to putative COOH-terminal βAPP derivatives previously described in human brain (*9*) and transfected cultured cells (*10–12*). Thus, the tris-tricine gel system was essential for demonstrating the complex set of COOH-terminal βAPP derivatives in human brain.

To determine whether some of the 8- to 12-kD COOH-terminal derivatives are large enough to contain the entire βAP, we compared these derivatives with a protein, produced by using rabbit reticulocyte lysate, that corresponds to the 100–amino acid segment at the βAPP COOH-terminus (*7*). This protein ($MβAP_1$-COOH) contains the entire βAP and begins with the methionine preceding the βAP sequence. $MβAP_1$-COOH comigrated with the second largest of the five COOH-terminal derivatives (Fig. 1E, lanes 1 and 2). Thus, comparative assessment by SDS-PAGE indicates that the ~11.8- and ~11.4-kD proteins are large enough to contain the entire βAP and therefore are potentially amyloidogenic.

To assess this possibility further, we used an antibody to $βAP_{1-40}$ (SGY2134) to immunoprecipitate COOH-terminal derivatives, which were assessed on immunoblots with anti-C_{20}. SGY2134 immunoprecipitated the two largest COOH-terminal derivatives but failed to immunoprecipitate the smaller COOH-terminal derivatives (Fig. 1F, lane 1). This immunoprecipitation was produced in large part by antibodies to the NH_2-terminal region of the βAP, because it was markedly attenuated by absorption with

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