HIGHLIGHTS OF BASIC SCIENCE RESEARCH

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Basic science contributions continue to represent an important and expanding feature of the Conference. New insight into the mechanism of action of the major regulatory protein, Tat, has been disclosed in the past year, and several presentations focused on the interaction of Tat with cellular proteins and how they impact on Tat function and the species-specific nature of Tat action. A number of presentations on the viral accessory gene products, particularly Vpr and Nef, revealed how these proteins influence the physiology of the target cell, and how these activities can influence the extent of virus replication and the ability of virus-infected cells to evade the immune response. Studies on chemokine receptors and how the level of chemokine-receptor expression influences viral transmission, tropism, and disease progression were featured in several sessions at the Conference. Biochemical and crystallographic analysis of viral structural proteins are leading to a better understanding of how these proteins mediate their action and ultimately may point to new approaches to inhibiting the action of the viral proteins.

ACCESSORY GENES

The accessory proteins of primate lentiviruses represent one of the most intensely studied areas of primate lentivirus biology, yet identification of the actual roles played by these proteins in primate lentiviral replication remains elusive. Inactivation of the accessory genes has been shown to impair viral replication and pathogenicity in infected monkeys to varying degrees. Thus, inactivation of *vif* has the greatest impact on viral replication followed by *nef*, *vpx*, *vpr*, and *vpu* genes. Despite the fact that these accessory proteins are important for virus replication in the host, the role played by these proteins in virus replication is not well understood. In addition, these proteins represent attractive targets for the development of antiviral drugs. Since the accessory proteins are unique to the virus and there are as yet no known homologous functions encoded by the cell, drugs targeted at viral accessory proteins would be predicted to have little effect on host cell function.

Nevertheless, the accessory proteins have not yet been exploited as drug targets, primarily because there are no convenient in vitro assays that reconstitute their activity and can be employed in large-scale inhibitor screens. One important area of investigation regarding accessory protein function regards cellular intermediaries through which these accessory proteins mediate their action, and the identification of such factors may be the best approach to identifying how the accessory proteins participate in virus replication.

One presentation **(Abstract 60)** demonstrated that mice transgenic for Nef developed tumors as early as 4 months. The majority of mice with tumors had adenocarcinoma. These studies are similar to studies published in 1998 by Jolicoeur and colleagues **(*Cell* 95:163, 1998)** that Nef was pathogenic in a transgenic mouse setting. It is unclear how these transgenic models relate to the action of *nef* in virus-infected humans and monkeys, but they may provide useful information on pathways through which *nef* mediates this effect. Although it is clear that *nef* facilitates virus replication and pathogenicity in the host, how Nef promotes these activities is not well understood.

Two well-recognized features of *nef* activity in vitro are the abilities of *nef* to downregulate the expression of CD4 and MHC Class I from the cell surface. Downregulation of CD4 by Nef may impact the virus life cycle at several levels. Infected cells with less cell surface CD4 may be resistant to superinfection of the same cell by progeny virions. Intracellular sequestration of CD4 may influence cell activation pathways through modulating interaction of CD4 with its associated kinase p56 Lck and, further, may restrict the incorporation of CD4 into virions during virus maturation. Surface expression of MHC Class I is required for efficient cytotoxic T lymphocyte (CTL) recognition of the infected cell. Through MHC Class I downregulation, *nef* may influence susceptibility of infected cells to CTL recognition, perhaps affording the virus a longer residence in the host cell before it is destroyed by CTL.

Research presented at the Conference **(Abstract S22)** examined the mechanism through which *nef* induces MHC Class I downregulation. New studies demonstrate that *nef* stimulates endocytosis of MHC Class I molecules, resulting in their degradation in lysosomes. This modulation was induced by Nef proteins from primary and laboratory-adapted HIV-1 isolates by HIV-2 and SIV-Nef proteins. *nef* was found to interact with μ chains of adaptor complexes of the endocytic pathway. By interacting with the cell trafficking machinery, *nef* induces MHC downregulation that, in turn, may provide some measure of immune escape to the infected cell.

In addition to CD4 and MHC Class I downregulation, *nef* has been reported to augment viral infectivity in single-cycle infectivity assays. Kotov and colleagues **(Abstract S21)** presented studies that suggest that *nef* is present in HIV-1 cores at levels similar to those in virions. One prediction of this result is that *nef*, as a component of the viral core, may influence early events in the viral life cycle, such as formation of the reverse transcription complex or synthesis of viral cDNA. Kotov et al also demonstrated that *nef* regulates the pathway of virus entry. Thus, deletion of *nef* facilitates the infection of cells through an endocytic pathway. This would suggest that one action of *nef* is to ensure that virus infection proceeds through pH-independent fusion.

Studies identifying regions of *nef* that were required for downregulation of CD4 were presented **(Abstract 510)**. A highly conserved sequence in the C-terminal loop of *nef* exhibiting homology to dileucine-based protein sorting-signals was found to be important for the ability of *nef* to promote CD4 downregulation. In addition, mutation of the dileucine motif in *nef* also impaired the infectivity enhancement by *nef*. This dileucine motif was found to be necessary for interaction between *nef* and a subset of adaptor protein complex subunits. Thus, while previous studies suggest CD4 downregulation and infectivity enhancement may be genetically separable, these activities may both depend on an interaction between *nef* and adaptor protein subunits. HIV-Nef and SIV-Nef contain motifs that promote interaction with Src homology 2 and Src homology 3 domains of Src family protein tyrosine kinases. *nef* has also been shown to interact with cellular serine/threonine kinases. The ability of *nef* to interact with kinase cascades has led to the suggestion that *nef* may influence cellular signaling/activation pathways of the cell.

Studies presented at the Conference **(Abstract 509)** demonstrated that HIV-1 *nef* interacts with PAK kinase in infected macrophages and, to a lesser extent, T lymphocytes. Though previous studies suggested that *nef* interacts with the myeloid specific Src kinase, Hck, investigators did not observe *Nef-Hck* interaction in primary macrophages. The consequences of *Nef-PAK* interaction in macrophage lineage cells will await further studies to determine how PAK influences macrophage function.

Most of the studies with the accessory gene products dealt with Vpr. To date, at least 4 properties for Vpr have been published, including (1) a role for Vpr in promoting infection of nondividing macrophages; (2) induction of cell cycle G2 arrest; (3) association of Vpr with the DNA repair enzyme, uracil DNA glycosylase (UDG); and (4) induction of cell differentiation.

Chen and colleagues **(Abstract S20)** presented evidence for a novel activity of Vpr. Their studies demonstrated that when cells were transfected with a plasmid that had been damaged by exposure to ultraviolet irradiation, the presence of Vpr in the transfected cell promoted DNA repair of the ultraviolet-damaged template. As yet, it is unclear how the presence of Vpr promotes the ability of the cell to repair damaged DNA, or whether this activity is related to one of the known properties of Vpr such as association with uracil DNA glycosylase.

Studies suggesting a role for Vpr in promoting fidelity of HIV-1 replication were discussed **(Abstract 512)**. Using a viral shuttle vector containing the lacZα peptide gene as a reporter gene for mutations, a panel of Vpr variants was examined for their effect on mutation rate when Vpr is expressed in trans. Mutations that influenced association of Vpr with UDG impaired the ability of Vpr to influence the mutation rate. This effect was, however, found to be independent of virion incorporation. These studies suggest that Vpr acts through UDG to preserve the fidelity of reverse transcription. It is unclear whether this effect is related to the effect of Vpr on DNA repair synthesis as reported at the Conference by Chen and colleagues **(Abstract S20)**.

Pavlakis and colleagues **(Abstract S23)** presented evidence that Vpr promotes activation of the glucocorticoid receptor promoter. Residues in Vpr were identified that inactivate the ability of Vpr to activate the glucocorticoid promoter. Promoter activation by Vpr was found to be independent of the ability of Vpr to induce cell cycle arrest. A coactivator motif (LXXLL), which mediates glucocorticoid receptor-promoter activation, was identified.

Pavlakis and colleagues also presented evidence that Vpr-green fluorescent protein (GFP) fusion proteins rapidly underwent nuclear translocation, an observation that is consistent with the role of Vpr in nuclear targeting of viral DNA in nondividing cells. One group **(Abstract 581)** provided evidence that phosphorylation of HIV-1 Vpr is important for Vpr function. Virus-infected cells labeled with 32P-orthophosphate released virions containing phosphorylated Vpr. Phosphoamino acid analysis of serine mutants suggested that S79, S94, and S96 were phosphorylated.

Studies to determine which of the Vpr functions rely on Vpr phosphorylation are ongoing. One feature of Vpr-induced G2 arrest is that this property can be reconstituted in fission yeast, thus demonstrating a high evolutionary conservation of function. Vpr properties of cell cycle arrest, nuclear localization, and cell death/differentiation were examined

in fission yeast using a panel of Vpr mutations. Induction of cell cycle arrest was independent of nuclear localization and of cell killing. Only Vpr alleles that localized to the nucleus exhibited cell killing. Thus, fission yeast may be a useful model system to evaluate Vpr functions and pathways through which Vpr mediates its effect.

Emerman and colleagues **(Abstract 580)** presented evidence that the HIV-1 long terminal repeat (LTR) was more active in the G2 phase of the cell cycle. This is consistent with a model in which Vpr-mediated delay of G2 progression may promote higher levels of virus production for each round of the cell cycle. HIV-1 virions that expressed Vpr alleles competent for G2 arrest also replicated to higher levels (3- to 5-fold) in primary T cells compared with viruses that lacked Vpr or that contained mutants of Vpr that did not cause G2 arrest. Thus, Vpr may delay cells at a stage in the cell cycle when the LTR is most active and in which virus production is maximal.

Emerman and colleagues also presented studies **(Abstract 511)** aimed at identifying the mechanism through which Vpr produces cell cycle arrest. Cell cycle progression is dependent on activation of the mitotic cyclin-dependent kinase, cyclin B, which normally controls the entry of cells into mitosis. Activation of this kinase requires removal of inhibitory phosphates on threonine 14 and tyrosine 15 by the phosphatase, CDC 25C. Emerman et al demonstrated that Vpr interacts with this phosphatase, thus preventing it from activating cyclin B and mutations in Vpr, which impaired its cell-cycle arrest capacity and also its ability to interact with CDC 25C.

Several studies expanded on the exciting demonstration in the past year of cellular cofactors that mediate Tat transactivation. Cullen and colleagues **(Abstract L3)** presented a model that Tat simply serves as a recruitment factor for cellular proteins that promote transactivation of the LTR. In the past year, Jones and colleagues demonstrated that human cyclin T1 and its associated kinase, CDK9, comprise a coactivator complex (***Cell* 92:451, 1998**). Human cyclin T1 interacts with the activation domain of Tat, and Tat recruits the cyclin T complex to the LTR where cyclin T1 activates HIV-1 LTR-directed transcription.

Cullen and colleagues also presented evidence **(Abstract 507)** that cyclin T1, when recruited via a heterologous RNA protein interaction, was able to activate the LTR independently of Tat. Thus, Tat simply acts as a recruitment factor that directs cyclin T1 to the LTR, but activation of transcription is independent of Tat. The ability of cyclin T1 to be recruited to Tar by Tat was also shown to determine the species restriction of HIV Tat. Thus, mouse cells do not support efficient Tat transactivation, because mouse cyclin T1 could not efficiently be recruited to Tar. Substitution of a single amino acid within mouse cyclin T1 reversed the species restriction and permitted efficient Tat transactivation in mouse cells expressing the mutant cyclin T1. These findings were echoed by Gaynor and colleagues **(Abstract S24)**. These studies provide new insight into the workings of Tat and point to novel targets that may be exploited therapeutically.

VIROLOGY

The Bernard Fields Memorial Lecture was given by Sodroski, who summarized the recent crystallographic characterization of HIV-1 envelope glycoproteins. These studies have a considerable impact on AIDS research in general because of their potential to yield insight into how CD4 and coreceptor binding expose fusogenic domains in envelope that may provide new targets for antiretroviral agents. In addition, studies invoking a central role for envelope glycoprotein in HIV-1-mediated pathogenesis were discussed.

Hahn and colleagues **(Abstract S2)** presented exciting studies aimed at uncovering the origin of HIV-1. All HIV-1 strains known to infect humans were found to be closely related to one of the known SIV CPZ lineages that originate in the chimpanzee, *Pan troglodytes troglodytes*. Hahn presented a model to suggest the mechanism for the zoonosis. The use of monkeys as a food source by humans likely precipitated exposure to infected animal tissue for generations. However, infection of humans likely remained geographically isolated until the "bush meat" trade resulted in widespread distribution of animal tissue to population centers. This may explain why SIV CPZ, which has existed in monkeys for millennia, may have emerged as a zoonosis within the last 40 to 50 years. Since SIV CPZ infection of monkeys is nonpathogenic, the interaction of the virus with its natural host will be critical to the understanding of how HIV-1 is pathogenic in its human host.

Ho and colleagues **(Abstract 10)** used plasma apheresis to influence the equilibrium between virus production and virus clearance. The magnitude of the decrease in plasma viremia during apheresis reflects the added clearance rate mediated by the apheresis procedure relative to the clearance mediated by viral clearance mechanisms in the host. Using an established mathematical model of viral dynamics, HIV particle half-life for 4 HIV-1-infected subjects was found to be between 39 and 109 minutes. In parallel, the half-life of hepatitis C virus was found to be 100 to 182 minutes. Daily virion produc-

tion estimates that were previously based on a virion half-life of 6 hours will therefore have to be revised accordingly. It is as yet unclear why these half-life estimates differ from those featured in a recent publication by Martin and colleagues (***Nature Medicine* 5:211, 1999**). In those studies, viral clearance was measured following infusion of virion particles into SIV-infected and uninfected monkeys and was in the order of several minutes. The differences probably reflect the distinct approaches taken by these groups to investigate the dynamics of viral clearance, such as a more rapid clearance of particles prepared ex vivo and then reinfused.

Sundquist and colleagues **(Abstract L5)** presented detailed electron microscopic studies of HIV-1 virion cores that had been reconstituted in vitro. RNA and capsid-nucleocapsid fusion proteins spontaneously assemble into conical particles that resemble authentic viral cores both in terms of size and morphology. The lattices that are formed in these synthetic cores resemble those exhibited by icosahedral viruses. Studies of this kind are critical to the development of assays that can be employed to screen for inhibitors of virion core assembly.

Several presentations focused on the viral Gag matrix protein. This structural virion protein has generated intense interest among investigators because it appears to exhibit novel activities in the viral life cycle. Viral matrix proteins are well recognized for their role in virus assembly and in maintaining the integrity of the intact virion. However, primate lentiviral matrix proteins have been implicated in several stages of the virus life cycle. Thus, Gag matrix has been shown to promote incorporation of envelope glycoproteins into the maturing virion. Intriguingly, the matrix protein has been shown to play a critical role in viral infectivity. Mutations in matrix that do not influence either envelope incorporation or virion maturation have been shown to block viral infectivity and, in addition, Gag matrix has been shown to promote nuclear translocation of the viral reverse transcription complex following infection of the target cell. Consistent with these activities, matrix has been shown to tightly associate with the reverse transcription complex.

Aiken and colleagues **(Abstract S21)** and Sundquist and colleagues **(Abstract L5)** presented evidence that Gag matrix is contained within viral cores, findings that would be consistent with its reported role in governing the function of the reverse transcription complex. However, the suggestion that Gag matrix is incorporated into virion cores was challenged by Göttlinger and colleagues who failed to observe the presence of Gag matrix in highly purified cores from HIV-1 infected cells **(Abstract 508)**. The mechanism through which Gag matrix influences viral infectivity remains controversial. Freed and colleagues **(Abstract S38)** identified mutations in matrix that disrupt an early postentry step in the virus life cycle. The defect was manifest at the level of reverse transcription, again suggesting an important role for matrix in maintaining the integrity and functioning of the reverse transcription complex.

Although matrix is an essential component of retroviral and lentiviral genomes, Göttlinger and colleagues **(Abstract S40)** presented evidence that in a specific T-cell line, virus replication can occur in the complete absence of matrix protein provided that concomitant deletions in the transmembrane glycoprotein of envelope were present. If HIV cores were pseudotyped with VSV-G envelope, matrix appeared to be dispensable for replication in T cells. In contrast, matrix-deletion mutants were impaired in their ability to infect nondividing macrophages, consistent with studies invoking a role for matrix in translocation of viral reverse transcription complexes in nondividing macrophages.

Several investigators presented studies that shed new insight into the role of specific genomic RNA encapsidation that is mediated by nucleocapsid. Summers and colleagues **(Abstract S37)** examined the basis through which RNA encapsidation is mediated by a specific interaction between nucleocapsid and a stem loop structure (SL3) in genomic RNA (known as the *psi* site). The tight interaction between nucleocapsid and SL3 RNA (K_d = 50 nM) was shown to be mediated by a specific interaction between zinc finger motifs of the nucleocapsid protein and G^7 and G^9 nucleotide bases of the G^6-G^7-A^8-G^9 RNA tetraloop. The NMR structure of NC protein bound to SL3 provides new insight into the mechanism of genomic RNA recognition and encapsidation. The process of RNA encapsidation ensures not only that unspliced genomic RNA specifically is incorporated into virions, but also that 2 copies of genomic RNA are incorporated into each viral particle. Wainberg and colleagues **(Abstract 513)** examined the phenotype of viruses containing mutations in an RNA stem loop structure implicated in dimerization of viral RNA. Deletions in this RNA dimerization site compromised viral replication in vitro. Long-term culture led to the emergence of revertant variants containing mutations in Gag matrix, Gag p2, and Gag nucleocapsid proteins. These findings may underscore additional roles for Gag matrix and Gag p2 in mediating either incorporation or dimerization of genomic viral RNA during virus assembly.

Given the well-recognized ability of HIV-1 to infect nondividing cells, a number of investigators have begun to

exploit HIV-1-based lentivirus vectors as a tool to transduce nondividing target cells. Although HIV-1-based vectors efficiently transduce nondividing cells such as macrophages, resting T cells are refractory to infection both by wild-type HIV and HIV base vectors. Rate-limiting levels of dNTPs, which are the building blocks for cDNA synthesis, and inefficient nuclear targeting of viral DNA have been implicated in the resistance of resting T cells to HIV-1 infection.

Littman and colleagues **(Abstract 56)** demonstrated that resting T cells could be transduced with an HIV-1-based vector if the cells were cultured in the presence of cytokines such as IL-2, IL-4, IL-7, or IL-15. Although it is unclear how this cytokine stimulation overcomes the block to infection of resting T cells by HIV-1, this approach will prove useful in transducing primary lymphocytes with HIV-1 without the need for additional exogenous stimuli.

The theme of resting cell infection by HIV-1 was echoed by Haase and colleagues **(Abstract LB4)** who examined the phenotype of SIV- and HIV-infected T cells in vivo. Following intravaginal inoculation of monkeys with SIV, most of the viral RNA-positive cells were found to be T cells both at the site of inoculation and in lymphatic tissues after dissemination. Surprisingly, however, most of the infected T cells did not appear to be activated since they did not express HLADR, Ki67, or cyclin A. These nonactivated cells expressed less viral RNA than infected cells that exhibited activation markers. Over the course of infection, the proportion of cells that were infected and activated increased. These studies suggest that T-cell activation may not be an absolute requirement for productive infection of T cells in vivo. It remains to be determined whether the productively infected nonactivated T cells in vivo resemble the cytokine-stimulated resting T cells that are permissive to productive HIV-1 infection as described by Littman and colleagues **(Abstract 56)**.

Viral Tropism/Coreceptor Usage

Research carried out over the past year has begun to enforce the model that CD4 does not serve as a viral receptor per se but promotes conformational changes in envelope that confer competence for coreceptor binding. In addition, HIV-1 and HIV-2 variants that do not require CD4 for infection have been described. This would suggest that envelope glycoproteins of these viruses are already in the appropriate configuration for coreceptor binding. One model proposed to explain the adaptation to CD4 utilization by primate lentiviruses invokes an "immune cloaking" mechanism. Presumably, coreceptor binding epitopes represent targets for neutralizing antibodies. Thus, in an attempt to avoid exposure of these epitopes, the virus has evolved to use CD4 interaction to expose coreceptor binding epitopes immediately prior to infection of the cell. A prediction of this model is that viruses that infect cells by a CD4-independent mechanism and that presumably have consistently exposed coreceptor binding epitopes may be more sensitive to antibody neutralization.

Consistent with this prediction, Doms and colleagues **(Abstract S11)** examined neutralization sensitivity of an HIV-1 isolate (HIV-1 IIIB8X), which utilizes CXCR4 without the need for CD4. Doms presented evidence that this variant was approximately 1 log more sensitive to neutralization by sera from HIV-1 seropositive individuals. Replacement of the V3 loop of the CXCR4-utilizing 8X virus with the V3 loop of a CCR5-utilizing virus resulted in a chimeric variant that exhibited CD4-independent CCR5-dependent infection. Thus, the V3 loop confers coreceptor choice but does not impact CD4 dependence. These studies point to new strategies for development of antibodies that target coreceptor binding epitopes of envelope glycoproteins, a theme that was mirrored by a recent publication in Science that fusion complexes derived from HIV-1 envelope expressing cells elicit broadly neutralizing antibody responses **(*Science* 283: 357, 1999)**.

As coreceptor ligands, SDF-1 (CXCR4 ligand) and RANTES, MIP-1α, and MIP-1β (CCR5 ligands) have been shown to inhibit HIV-1 infection. Studies presented at the Conference **(Abstracts S12, S13)** demonstrated paradoxically that RANTES enhances infectivity of HIV-1 isolates via CXCR4 (X4 isolates). Trkola and colleagues **(Abstract S13)** describe the mechanism through which RANTES enhances virus infection. RANTES was shown to enhance not only HIV-1 infectivity but also the infectivity of other viruses such as vaccinia, influenza, VSV, and MLV. Thus, the RANTES enhancement effect appeared to be independent of the route of virus entry or of coreceptor usage. Two mechanisms of enhancement were suggested. The first involves a mechanism in which RANTES may induce signaling and alter permissivity of cells to virus entry. A second mechanism likely involves cross-linking of virions to cells through interaction of RANTES with glycosoaminoglycans on cell and viral membranes that could be inhibited by treatment of cells with chondroitin sulfate.

Gordon and colleagues **(Abstract 505)** echoed the finding that RANTES enhances viral infectivity by 2 mechanisms that are inde-

pendent of the mode of viral entry or coreceptor usage. Sodroski and colleagues **(Abstract S13A)** demonstrated that posttranslational modifications on CCR5 modulate the ability of these proteins to mediate HIV-1 entry. CCR5 was shown to be modified by O-glycosylation and by sulfation of its N-terminal tyrosines. Sulfated tyro-sines modulated the binding of CCR5 to MIP-1α and MIP-1β and to gp120/CD4 complexes. Mutation of a critical sulfated tyrosine impaired the ability of HIV-1 to enter cells via CCR5 and CD4. The authors suggested that differences in CCR5 sulfation between different cell types would impact on the ability of these cells to be infected by HIV via CCR5.

In approximately 50% of patients who progress to full-blown AIDS, there is an apparent switch in coreceptor usage in that viruses obtained late in disease exhibit CXCR4 (X4) tropism whereas viruses obtained early in disease exhibit predominantly CCR5 (R5) tropism. Studies presented at the Conference **(Abstract 521)** suggested that envelope sequences derived from HIV-1 isolates obtained from both brain and colon exhibited R5 usage, not X4 usage. Thus, in contrast to the frequently observed switch in coreceptor specificity in comparing blood-derived viruses early after HIV infected and after the onset of AIDS, CCR5 appeared to be a primary coreceptor for brain-derived and colon-derived viruses. Thus, tissue infection appears to select for CCR5 usage throughout disease progression.

HIV-1 infection of macrophages is mediated by CCR5. Thus, macrophages obtained from individuals with a homozygous deletion in CCR5 are resistant to infection by macrophage tropic HIV-1. It has also been established that while CXCR4 is expressed on macrophages, it is inefficiently utilized by X4 tropic viruses. Studies presented at the Conference **(Abstract 499)** further investigated the mechanism through which some X4 tropic viruses are able to utilize CXCR4 for infection of macrophages. The investigators analyzed a dual tropic isolate (DH12), which uses CCR5 and CXCR4 and which infects macro-phages from homozygous CCR5 deleted individuals. The investigators further compared macrophage infectivity by lab-adapted and primary isolates that exhibit X4 tropism. Primary HIV-1 isolates were able to infect macrophages via CXCR4, which is in contrast to lab-adapted strains that were not able to utilize CXCR4 for macrophage infection. Thus, the failure of lab-adapted isolates to enter macrophages through CXCR4 may be a feature of lab adaption and primary dual tropic viruses may exploit both coreceptors for macrophage infection. ■

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