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VIRUS-CELL INTERACTIONS

Functional Evidence of the Involvement of the Dynein Light Chain DYNLRB2 in Murine Leukemia Virus Infection

Tatiana Opazo, Andrea Garcés, Diego Tapia, Felipe Barraza, Angélica Bravo, Tomás Schwenke, Jorge Cancino, Gloria Arriagada

Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello, Viña del Mar, Chile

ABSTRACT How murine leukemia virus (MLV) travels from the cell membrane to the nucleus and the mechanism for nuclear entry of MLV DNA in dividing cells still remain unclear. It seems likely that the MLV preintegration complex (PIC) interacts with cellular proteins to perform these tasks. We recently published that the microtubule motor cytoplasmic dynein complex and its regulator proteins interact with the MLV PIC at early times of infection, suggesting a functional interaction between the incoming viral particles, the dynein complex, and dynein regulators. To better understand the role of the dynein complex in MLV infection, we performed short hairpin RNA (shRNA) screening of the dynein light chains on MLV infection. We found that silencing of a specific light chain of the cytoplasmic dynein complex, DYNLRB2, reduced the efficiency of infection by MLV reporter viruses without affecting HIV-1 infection. Furthermore, the overexpression of DYNLRB2 increased infection by MLV. We conclude that the DYNLRB2 light chain of the cytoplasmic dynein complex is an important and specific piece of the host machinery needed for MLV infection.

IMPORTANCE Retroviruses must reach the chromatin of their host to integrate their viral DNA, but first they must get into the nucleus. The cytoplasm is a crowded environment in which simple diffusion is slow, and thus viruses utilize retrograde transport along the microtubule network, mediated by the dynein complex. Different viruses use different components of this multisubunit complex. We have found that murine leukemia virus (MLV) associates functionally and specifically with the dynein light chain DYNLRB2, which is required for infection. Our study provides more insight into the molecular requirements for retrograde transport of the MLV preintegration complex and demonstrates, for the first time, a role for DYNLRB2 in viral infection.

KEYWORDS MLV, dynein, dynein light chain, DYNLRB2

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Address correspondence to Gloria Arriagada, gloria.arriagada@unab.cl.

T.O. and A.G. contributed equally to this article.

During early steps of infection, retroviruses must reach the nucleus, cross the nuclear membrane, and access the host cell genome. The cytoplasm is extremely crowded, and simple diffusion is predicted to be too slow to explain the kinetics of virus infection (1). Many retroviruses are actively transported along microtubules (2–4) to move through the cytoplasm and reach the nucleus, and the microtubule retrograde transport complex dynein has been shown to have an important role in trafficking and infection for several viral families (2, 5–16).

Murine leukemia virus (MLV) belongs to the *Gammaretrovirus* genus, whose members are strongly dependent on cell division to gain entry into the nucleus. These viruses do not have the ability to cross the nuclear membrane of nondividing cells and rather must wait for nuclear membrane breakdown to occur during mitosis to access host chromatin. Several screens have revealed that cytoskeleton-associated proteins

are important in different stages of retrovirus replication (8, 17). Following that line, we identified dynein and its regulators as important parts of the host cell machinery required for MLV infection (16), with the dynein regulators seeming to be essential for a step before nuclear entry but after reverse transcription. We also reported that the dynein intermediate chain is important for MLV infection, since its knockdown significantly reduced MLV infection.

Dynein, the motor associated with microtubule minus-end transport, is a multisubunit complex composed of two copies of the heavy chain (DYNC1H1), two intermediate chains (DYNC1I1/2), two light-intermediate chains (DYNC1LI1/2), and two copies of three different light chains (DYNLT1/3, DYNLRB1/2, and DYNLL1/2). Heavy chains are the ATPase motors that bind to microtubules and generate the mechanical force for movement, while the other subunits acts as regulators and adaptors to different cargoes (reviewed in reference 18). The intermediate chain mediates not only cargo association with the dynein complex but also the association of the light-intermediate and light chains with the heavy chain. Some retroviruses, such as bovine immunodeficiency virus (BIV), Mason-Pfizer monkey virus (MPMV), prototype foamy virus (PFV), and human immunodeficiency virus type 1 (HIV-1), interact directly with the cytoplasmic dynein light chains for their retrograde transport (13, 15, 19–21). Thus, the association of retroviruses with dynein light chains seems to be a common mechanism. We hypothesized that MLV uses the same mechanism, associating functionally with a specific dynein light chain. To probe this, we performed a short hairpin RNA (shRNA) screening of the dynein light chains and found that MLV indeed follows the line of other retroviruses and functionally associates with dynein light chains. Unlike other viruses, though, MLV specifically requires the DYNLRB2 chain of the dynein complex. Therefore, although the retrograde movements of retroviruses or any virus use a common mechanism, association with the dynein complex, each particular virus apparently uses a specific dynein chain.

RESULTS

We recently demonstrated that the MLV preintegration complex (PIC) associates with dynein and dynein regulators (16); specifically, the dynein intermediate chain DYNC1I2 is required for MLV infection. The dynein intermediate chain is not only important for cargo binding but also essential for the association of dynein light chains with the complex (22). Considering that dynein light chains have an important role in MPMV, BIV, PFV, and HIV-1 infections (9, 13, 15, 21), we decided to explore the possible role of the different light chains of cytoplasmic dynein in the early events of MLV infection. We generated stable knockdown (KD) cells by using shRNAs against each dynein light chain and then challenged the cells with MLV reporter viruses. The dynein light chains are essential for cell survival, as they have important roles in the maintenance of the cytoskeleton, organelle positioning, and cell division (reviewed in references 22 and 23). For this reason, we selected for cells that produced sufficient quantities of these proteins to survive and function in the long term but had reduced mRNA levels compared to those in nonsilenced stable cell lines.

KD of DYNLL1 (Fig. 1A, gray bars) did not have a significant effect on ecotropic MLV infection (Fig. 1B; also see Fig. 3D). Similarly, KD of DYNLL2 (Fig. 1A, black bar) did not affect MLV infection (Fig. 1C; also see 3D). These results indicate that neither DYNLL1 nor DYNLL2 is required for MLV infection.

KD of DYNLT1 (Fig. 2A, gray bar) did not affect MLV infection (Fig. 2B), and KD of DYNLT3 (Fig. 2A, black bars) also showed no difference in MLV infection compared to that of the nonsilenced cell line (Fig. 2C). The overall infection of MLV in DYNLT1 or DYNLT3 KD cells was not significantly different from that in the nonsilenced cell line (Fig. 3D). Therefore, the DYNLT chains are not required for MLV infection.

Strikingly, when DYNLRB1 KD cells (Fig. 3A, gray bars) were infected, we observed a much higher infection level than that with nonsilenced cells (Fig. 3B), with up to a 2.5-fold increase (Fig. 3D). In contrast, knockdown of DYNLRB2 (Fig. 3A, black bars) significantly reduced MLV infection (Fig. 3C and D). These results point to the possibility

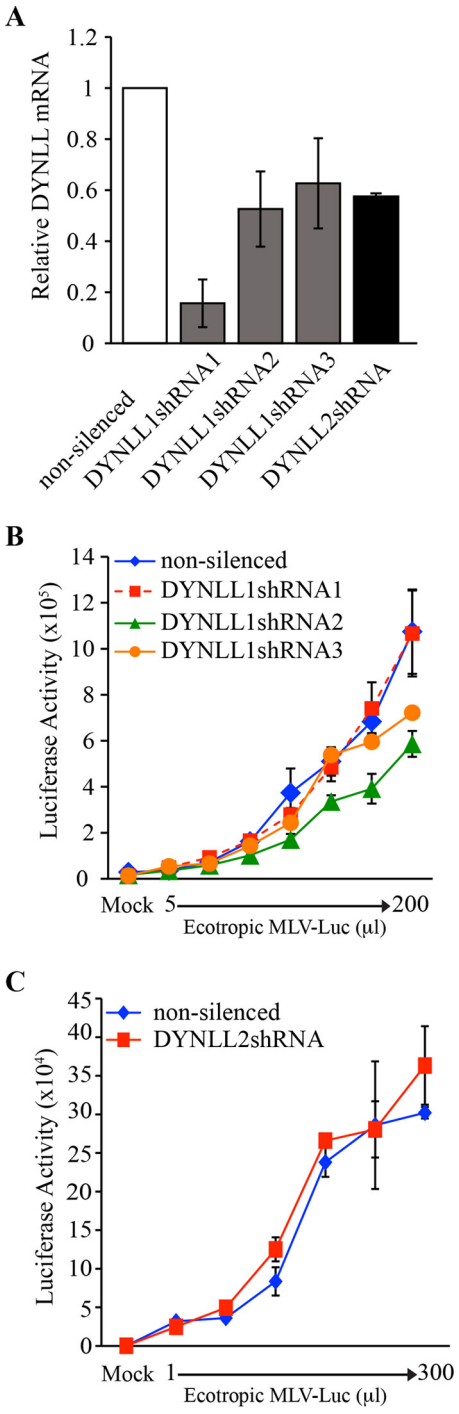


FIG 1 Cytoplasmic dynein light chains DYNLLs do not participate in MLV infection. (A) DYNLL1 and DYNLL2 mRNA levels in cells engineered by DYNLL1 or DYNLL2 KD were determined by quantitative PCR. The values were normalized to CypA mRNA and expressed relative to the nonsilenced control level. (B) Control and DYNLL1 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. (C) Control and DYNLL2 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. Forty-eight hours after infection, luciferase activity was measured. Data from one representative of three independent experiments are shown. Error bars indicate standard deviations between triplicates in the same experiment.

that the DYNLRB1 KD cells were compensating for the lack of DYNLRB1 by increasing the level of DYNLRB2. In fact, we observed that DYNLRB2 mRNA was increased >2.5-fold in the DYNLRB1 KD cells (Fig. 3E). To verify that the overexpression of DYNLRB2 was the cause of increased infection and to discard an off-target effect of the

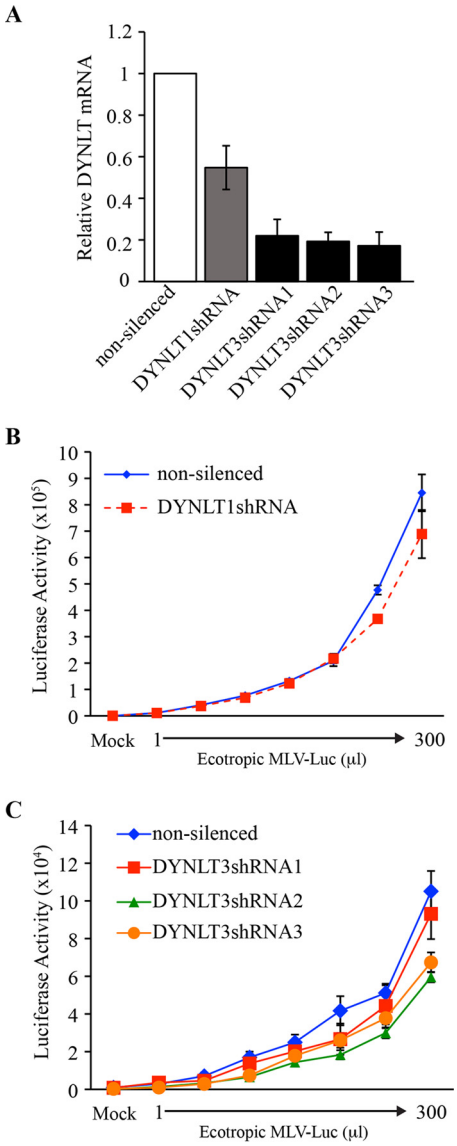


FIG 2 Cytoplasmic dynein light chains DYNTs are not required for MLV infection. (A) DYNLT1 and DYNLT3 mRNA levels in cells engineered by DYNLT1 or DYNLT3 KD were determined by quantitative PCR. The values were normalized to CypA mRNA and expressed relative to the nonsilenced control level. (B) Control and DYNLT1 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. (C) Control and DYNLT3 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. Forty-eight hours after infection, luciferase activity was measured. Data from one representative of three independent experiments are shown. Error bars indicate standard deviations between triplicates in the same experiment.

shRNAs, we transfected the control nonsilenced and DYNLRB2 KD cells with a hemagglutinin-DYNLRB2 (HA-DYNLRB2)-expressing construct. The overexpression of DYNLRB2 in the nonsilenced cells robustly increased infection by MLV (Fig. 3F, blue bars); strikingly, not only were the DYNLRB2 KD cells infected by MLV, but the level of infection went above the levels in the nonsilenced cell line transfected with the empty vector, despite the fact that HA-DYNLRB2 was also a target of the shRNAs. Altogether, these results demonstrate that DYNLRB2 is an important cellular factor for MLV infection.

In order to exclude the possibility that the reduced infection of MLV upon KD of DYNLRB2 was due to a broader effect on the cells, we analyzed the localization and dynamics of lysosomes, which are affected when dynein activity is inhibited (12). We

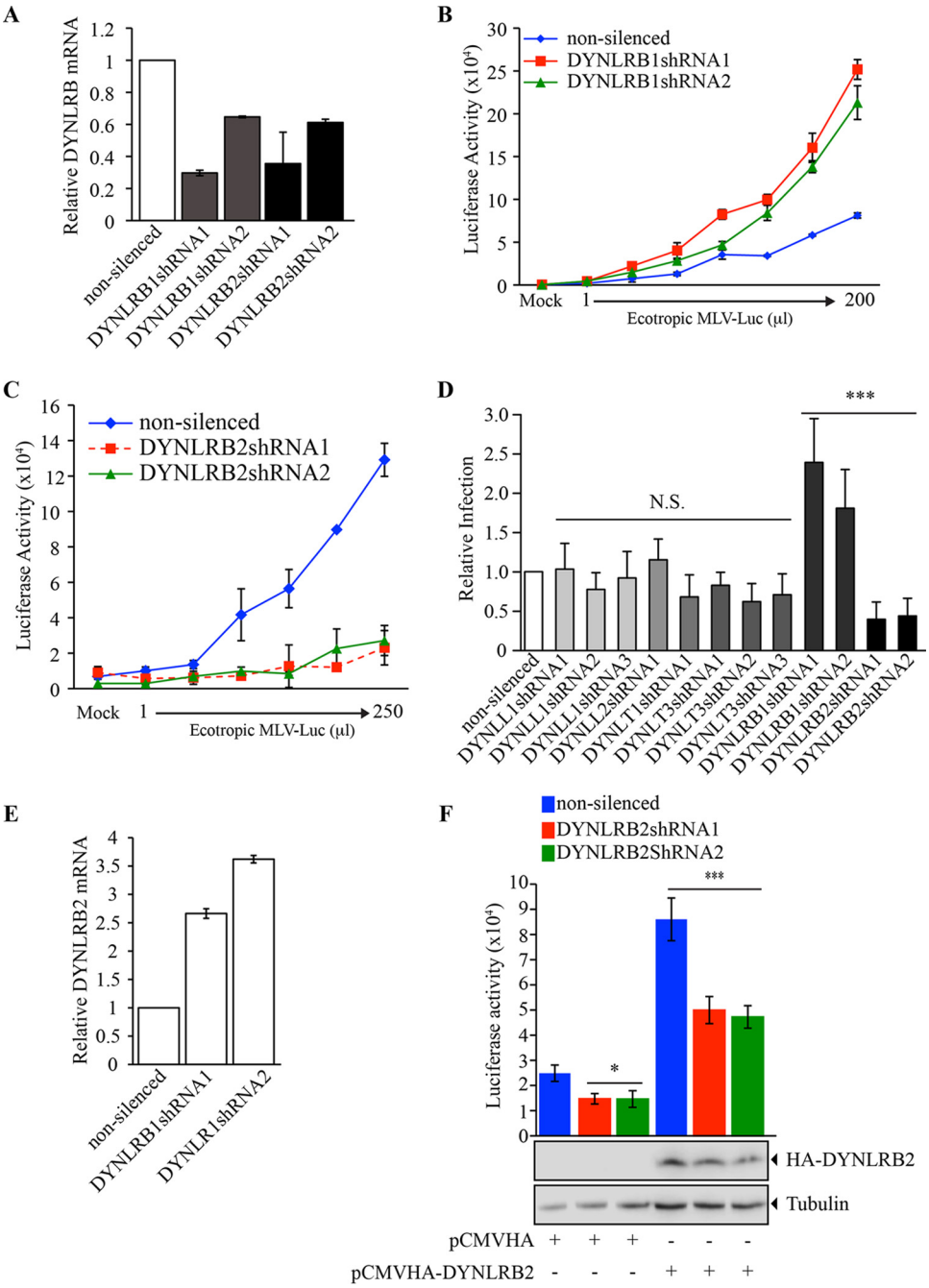
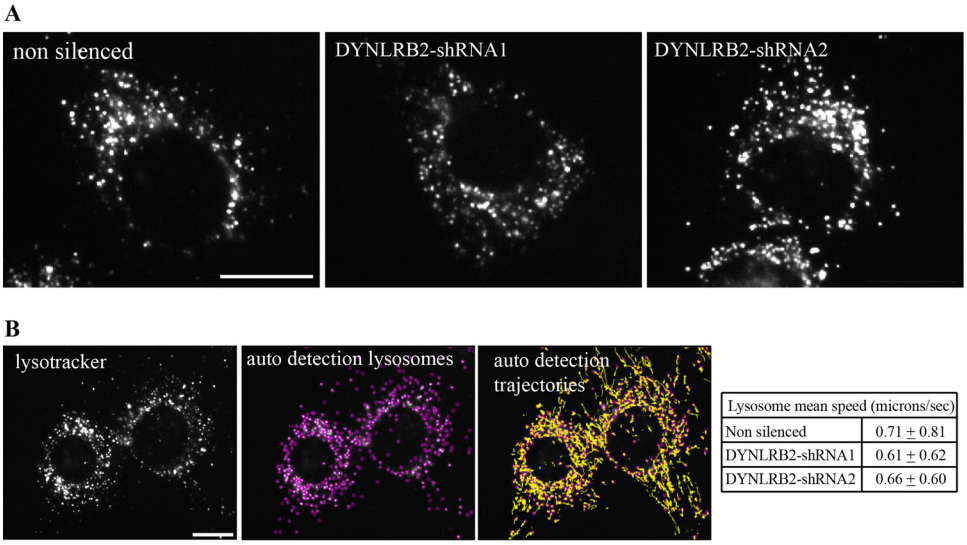


FIG 3 Cytoplasmic dynein light chain DYNLRB2 is required for MLV infection. (A) DYNLRB1 and DYNLRB2 mRNA levels in cells engineered by DYNLRB1 or DYNLRB2 KD were determined by quantitative PCR. The values were normalized to CypA mRNA and expressed relative to the nonsilenced control level. (B) Control and DYNLRB1 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. (C) Control and DYNLRB2 KD cells were challenged with increasing amounts of an ecotropic MLV luciferase reporter virus. Forty-eight hours after infection, luciferase activity was measured. Data from one representative of three independent experiments are shown. Error bars indicate standard deviations between triplicates in the same experiment. (D) Relative infection levels of the ecotropic MLV luciferase reporter virus in DYNLL1, DYNLL2, DYNLT1, DYNLT3, DYNLRB1, and DYNLRB2 KD cells compared to that in nonsilenced control cells. Error bars represent the standard deviations among the 3 independent experiments. N.S., not significant; ***, $P < 0.001$. (E) DYNLRB2 mRNA levels in cells engineered by DYNLRB1 KD were determined by quantitative PCR. The values were normalized to CypA mRNA and expressed relative to the nonsilenced control level. (F) Nonsilenced control cells and DYNLRB2 KD cells were transfected with a plasmid encoding HA-DYNLRB2 or with empty vector, and 24 h later they were challenged with a single dose of ecotropic MLV luciferase reporter virus. Forty-eight hours after infection, luciferase activity was measured. Data from one representative of three independent experiments are shown. Error bars indicate standard deviations between triplicates in the same experiment. The presence of HA-DYNLRB2 was determined by Western blotting, using tubulin as a loading control. *, $P < 0.05$; ***, $P < 0.001$.



Lysosome mean speed (microns/sec)	
Non silenced	0.71 ± 0.81
DYNLRB2-shRNA1	0.61 ± 0.62
DYNLRB2-shRNA2	0.66 ± 0.60

FIG 4 Lysosomal localization and dynamics are not affected by DYNLRB2 KD. Control and DYNLRB2 KD cells were incubated with LysoTracker. (A) Localization of lysosomes. (B) Dynamics of lysosomes. Lysosomes (purple circles) and lysosome trajectories (yellow tracks) were identified by LoG Detector and Simple LAP Tracker, respectively. The mean (± standard deviation) lysosome speed was calculated from 3 independent experiments and is shown in micrometers per second. Bar, 10 μm.

observed that both lysosomal localization (Fig. 4A) and dynamics (Fig. 4B) were similar among nonsilenced and DYNLRB2 KD cells. We also determined the duplication times of the nonsilenced and DYNLRB2 KD cells. We found that the duplication rate over a 48-h period for the nonsilenced cells (1.37 ± 0.21) did not differ from that for the DYNLRB2 KD cells (1.25 ± 0.12 and 1.3 ± 0.24 for shRNA1 and shRNA2, respectively). These results indicate that the general health of the DYNLRB2 KD cells was similar to that of the nonsilenced cells.

We previously showed that dynactin components have a differential effect on the different tropisms of MLV: ecotropic MLV strongly requires them, while amphotropic MLV only mildly requires them (16). Therefore, we tested if infections by amphotropic and vesicular stomatitis virus glycoprotein G (VSV-G)-pseudotyped MLV strains were or were not reduced in DYNLRB2 KD cells. We observed that infections by the amphotropic (Fig. 5A) and VSV-G-pseudotyped (Fig. 5B) MLVs tested were reduced in DYNLRB2 KD cells. In order to test if the dependence on DYNLRB2 was particular to MLV, we also challenged the DYNLRB2 KD cells with an ecotropic version of HIV-1 (Eco-HIV), and we observed that infections by Eco-HIV proceeded similarly in both nonsilenced and DYNLRB2 KD cells. This result confirms that the general health of cells is not affected upon DYNLRB2 KD and indicates that DYNLRB2 is essential for MLV infection but not for HIV-1 infection.

DISCUSSION

Our study provides new insight into the requirements for retrograde transport of the MLV preintegration complex and demonstrates, for the first time, a role for DYNLRB2 in viral infection. We previously showed that the intermediate chain DYNC1I2 of the dynein complex associates with the MLV PIC and that KD of DYNC1I2 in NIH 3T3 cells reduces infection by MLV (16). This phenotype may be associated directly with a lack of interaction between MLV and the dynein intermediate chain, with the reduced association of other important factors, such as p50 or NudEL, with the dynein complex, or with a reduced association of other dynein chains with the dynein complex. We tested the last possibility based on the facts that the dynein intermediate chain, besides its cargo binding function, is the anchor for the light-intermediate and light chains of the dynein complex (reviewed in references 22 and 24) and that several viruses, including retroviruses, associate with dynein light chains (reviewed in reference 25).

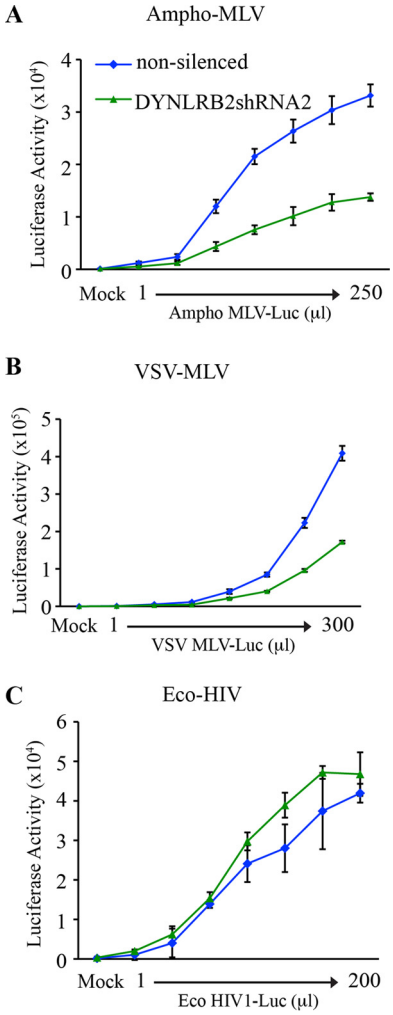


FIG 5 MLV infection requires DYNLRB2 independently of the entry pathway. (A and B) Control and DYNLRB2 KD cells were challenged, in parallel, with increasing amounts of an amphotropic (A) or VSV-G-pseudotyped (B) MLV luciferase reporter virus. (C) Control and DYNLRB2 KD cells were challenged with increasing amounts of an ecotropic pseudotyped HIV-1 luciferase reporter virus. Forty-eight hours after infection, luciferase activity was measured. Data from one representative of four independent experiments are shown. Error bars indicate standard deviations between triplicates in the same experiment.

Our main candidates were the DYNLL1/2 proteins. DYNLL or LC8 has been involved in foamy virus association with centrosomes, mediated by a direct interaction between Gag and DYNLL (13). By use of a *Saccharomyces cerevisiae* model, it has been shown that HIV-1 IN associates with Dyn2p, a DYNLL ortholog (21), and a functional interaction between DYNLL1 and HIV-1 has also been demonstrated (9). In the case of BIV, DYNLL interacts directly with the CA proteins, and this interaction mediates retrograde transport of the incoming virus (15). Moreover, DYNLL binding motifs have been found in several viral proteins (26). Unlike what we expected, neither KD of DYNLL1 nor that of DYNLL2 affected MLV infection, demonstrating that the DYNLL proteins are not required for MLV infection.

The next candidates were the DYNLT1/3 proteins. DYNLTs have also been associated with infections by several viruses, such as human papillomavirus (14), flavivirus (27), and retroviruses. Mason-Pfizer monkey virus requires DYNLT1 for the localization of viral proteins to assembly sites (19), and the nuclear localization of the HIV-1 accessory gene *vpr* is dependent on its interaction with DYNLT1 (20). Although we observed a small reduction of MLV infection in DYNLT1 and DYNLT3 KD cells, this was not significant, indicating that like DYNLLs, DYNLTs do not play an important role in MLV infection.

Unexpectedly, DYNLRB2 is the light chain required for MLV infection. KD of DYNLRB2 reduces MLV infection, while increased expression of DYNLRB2 increases the level of infection. Until now, the association (functional or physical) between dynein light chains and viruses was restricted to the DYNLL and DYNLT chains. Therefore, this is the first report describing a function for DYNLRBs in viral infection. We thought that, as with the other light chains, it would be possible to demonstrate a direct interaction between DYNLRB2 and viral proteins, but after several attempts using recombinant p12, CA, IN, and DYNLRB2 produced in bacteria or p12, CA, and IN from bacteria and DYNLRB2 produced in eukaryotic cells, we have not been able to observe such an interaction. It is possible that DYNLRB2 does not interact directly with those proteins and rather associates with the PIC or with one of those proteins in the context of the PIC. It is also possible that another protein associated with DYNLRB2 is the link between the MLV PIC and the dynein complex. Regardless of the lack of evidence for a physical interaction between DYNLRB2 and MLV proteins, a functional association between MLV and DYNLRB2 is in line with the idea that each particular virus requires a particular component of the dynein complex.

The requirement of DYNLRB2 for MLV infection was independent of the envelope used in the assays. This is different from what we previously observed for dynein regulators (16) and indicates that the functional association between MLV and DYNLRB2 is important in a step after the exit of the incoming particle from the endocytosed vesicle. Importantly, this is not a general effect on the cell, as otherwise we would have observed an alteration in the localization and dynamics of lysosomes and a reduction in HIV-1 infection, and that was not the case. The last observation also suggests that DYNLRB2 might be used only for gammaretroviruses, if not exclusively for MLV.

Since MLV requires the breakdown of the nuclear envelope for nuclear entry, DYNLRB2 might help to bring the PIC into close proximity with the nucleus and to associate it with mitotic chromosomes. We are currently testing if that is the case.

This study provides new insight into the cellular requirements of MLV infection and demonstrates a role for DYNLRB2 in viral infection.

MATERIALS AND METHODS

Cell lines. Human embryonic fibroblasts (293T) and mouse fibroblasts (NIH 3T3) were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 mg/ml streptomycin. All cells were cultured at 37°C in 5% CO_2.

Plasmids. Plasmid DNAs were as follows. pCMVI expresses *gag* and *pol* from NB-MLV. pHit123 expresses the ecotropic envelope of MLV. pHit456 expresses the amphotropic envelope of MLV. pFBLuc (Stratagene) is a reporter plasmid containing the firefly luciferase coding sequence flanked by MLV-based long terminal repeats (LTRs). p8.91 carries *gag* and *pol* of HIV-1. pMD.G expresses the vesicular stomatitis virus envelope glycoprotein. pNCS contains the full-length Moloney murine leukemia virus. pNL43lucΔenv encodes an envelope-deficient HIV-1 strain with a luciferase reporter gene. pCMVHA-DYNLRB2 expresses the mouse DYNLRB2 protein fused to an HA epitope.

Generation of stable cell lines. Lentiviruses for transduction were produced by transfection of 293T cells with a mixture of the following plasmid DNAs: 1 μg pMD.G, 1 μg p8.91, and 1.5 μg of pGIPz, pGIPzDYNLL1 (containing shRNAs 1 to 3), pGIPzDYNLL2 (containing shRNA 1), pGIPzDYNLT1 (containing shRNA 1), pGIPzDYNLT3 (containing shRNAs 1 to 3), pGIPzDYNLRB1 (containing shRNAs 1 and 2), or pGIPzDYNLRB2 (containing shRNAs 1 and 2) (Open Biosystems). Viruses were harvested 48 h after infection, filtered (0.45 μm), and used to infect 5×10^4 NIH 3T3 cells in 35-mm dishes in the presence of 8 μg/ml Polybrene. Cells were selected in 1 μg/ml puromycin.

Analysis of knockdown. Cells were harvested, and total RNA was extracted using TRIzol reagent (Invitrogen). Two micrograms of total RNA per cell line was used in reverse transcription reaction mixtures to produce cDNAs by use of random hexamers and an Affinity Script qPCR cDNA synthesis kit (Agilent Technologies). Two microliters of each cDNA was used for qPCR analysis of DYNLL1, DYNLL2, DYNLT1, DYNLT3, DYNLRB1, DYNLRB2, and CypA transcript levels. Fold changes were calculated using the relative standard curve method.

Single-cycle infectivity assay. NB luciferase reporter viruses were produced by transfection of 293T cells with 10 μg pCMVI, 10 μg pHIT123, pHIT456, or pMDG, and 20 μg pFBluc (per 100-mm plate) by use of calcium phosphate. An ecotropic HIV-1 reporter virus was produced by transfection of 293T cells with 10 μg pHIT123 and 20 μg pNL43-lucΔenv. Reporter virus stocks were harvested 48 h after transfection, filtered (0.45 μm), and stored at −80°C. NIH 3T3 cells (2.5×10^4 per well) were seeded in 24-well plates and infected with MLV luciferase reporter viruses. At 48 h postinfection, cells were collected and assayed for firefly luciferase activity (Promega) in a luminometer. For the phenotype recovery assay, DYNLRB2 KD

cells (2.5×10^4 per well) were seeded in 12-well plates and transfected with 0.25 μg of pCMVHA or pCMVHA-DYNLRB2, and 24 h after transfection the cells were infected as described above.

Western blotting. Cells were lysed in reporter lysis buffer (Promega). Samples were then boiled in 5× sodium dodecyl sulfate (SDS) loading buffer, and the proteins were resolved in a Novex 16% Tricine protein gel (Thermo Fisher). After transfer to nitrocellulose membranes, the blots were probed with mouse anti-HA (clone 16B12; Covance) and mouse anti-tubulin (Sigma). Secondary antibodies conjugated to horseradish peroxidase (HRP) and enhanced chemiluminescence (ECL) reagents were used for development.

Lysosomal localization and dynamics. NIH 3T3 nonsilenced and DYNLRB2-silenced cells were incubated with LysoTracker in DMEM with 10 mM HEPES for 5 min at 37°C. After incubation, fresh red phenol-free DMEM with 10 mM HEPES was added, and lysosomal localization and dynamics were recorded by time-lapse imaging at 37°C. Images were acquired with a Leica DMIL microscope mounted with a Leica DFC3000G monochrome camera, using LASX Leica software. Images were analyzed with ImageJ (Fiji) running the TrackMate (v2.8.1) plug-in.

Statistical analysis. All statistical analyses were performed using one-way analysis of variance (ANOVA) with a 95% confidence level in GraphPad Prism.

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REFERENCES

1. Luby-Phelps K. 2000. Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area. Int Rev Cytol 192:189–221.
2. McDonald D, Vodicka MA, Lucero G, Svitkina TM, Borisy GG, Emerman M, Hope TJ. 2002. Visualization of the intracellular behavior of HIV in living cells. J Cell Biol 159:441–452. https://doi.org/10.1083/jcb.200203150.
3. Sabo Y, Walsh D, Barry DS, Tinaztepe S, de Los Santos K, Goff SP, Gundersen GG, Naghavi MH. 2013. HIV-1 induces the formation of stable microtubules to enhance early infection. Cell Host Microbe 14:535–546. https://doi.org/10.1016/j.chom.2013.10.012.
4. Campbell EM, Hope TJ. 2008. Live cell imaging of the HIV-1 life cycle. Trends Microbiol 16:580–587. https://doi.org/10.1016/j.tim.2008.09.006.
5. Bremner KH, Scherer J, Yi J, Vershinin M, Gross SP, Vallee RB. 2009. Adenovirus transport via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. Cell Host Microbe 6:523–535. https://doi.org/10.1016/j.chom.2009.11.006.
6. Engelke MF, Burckhardt CJ, Morf MK, Greber UF. 2011. The dynactin complex enhances the speed of microtubule-dependent motions of adenovirus both towards and away from the nucleus. Viruses 3:233–253. https://doi.org/10.3390/v3030233.
7. Florin L, Becker KA, Lambert C, Nowak T, Sapp C, Strand D, Streeck RE, Sapp M. 2006. Identification of a dynein interacting domain in the papillomavirus minor capsid protein L2. J Virol 80:6691–6696. https://doi.org/10.1128/JVI.00057-06.
8. Gallo DE, Hope TJ. 2012. Knockdown of MAP4 and DNAL1 produces a post-fusion and pre-nuclear translocation impairment in HIV-1 replication. Virology 422:13–21. https://doi.org/10.1016/j.virol.2011.09.015.
9. Jayappa KD, Ao Z, Wang X, Mouland AJ, Shekhar S, Yang X, Yao X. 2015. Human immunodeficiency virus type 1 employs the cellular dynein light chain 1 protein for reverse transcription through interaction with its integrase protein. J Virol 89:3497–3511. https://doi.org/10.1128/JVI.03347-14.
10. Lukic Z, Dharan A, Fricke T, Diaz-Griffero F, Campbell EM. 2014. HIV-1 uncoating is facilitated by dynein and kinesin 1. J Virol 88:13613–13625. https://doi.org/10.1128/JVI.02219-14.
11. Merino-Gracia J, Garcia-Mayoral MF, Rodriguez-Crespo I. 2011. The association of viral proteins with host cell dynein components during virus infection. FEBS J 278:2997–3011. https://doi.org/10.1111/j.1742-4658.2011.08252.x.
12. Pawlica P, Berthoux L. 2014. Cytoplasmic dynein promotes HIV-1 uncoating. Viruses 6:4195–4211. https://doi.org/10.3390/v6114195.
13. Petit C, Giron ML, Tobaly-Tapiero J, Bittoun P, Real E, Jacob Y, Tordo N, De The H, Saib A. 2003. Targeting of incoming retroviral Gag to the centrosome involves a direct interaction with the dynein light chain 8. J Cell Sci 116:3433–3442. https://doi.org/10.1242/jcs.00613.
14. Schneider MA, Spoden GA, Florin L, Lambert C. 2011. Identification of the dynein light chains required for human papillomavirus infection. Cell Microbiol 13:32–46. https://doi.org/10.1111/j.1462-5822.2010.01515.x.
15. Su Y, Qiao W, Guo T, Tan J, Li Z, Chen Y, Li X, Li Y, Zhou J, Chen Q. 2010. Microtubule-dependent retrograde transport of bovine immunodeficiency virus. Cell Microbiol 12:1098–1107. https://doi.org/10.1111/j.1462-5822.2010.01453.x.
16. Valle-Tenney R, Opazo T, Cancino J, Goff SP, Arriagada G. 2016. Dynein regulators are important for ecotropic murine leukemia virus infection. J Virol 90:6896–6905. https://doi.org/10.1128/JVI.00863-16.
17. Naghavi MH, Valente S, Hatziioannou T, de Los Santos K, Wen Y, Mott C, Gundersen GG, Goff SP. 2007. Moesin regulates stable microtubule formation and limits retroviral infection in cultured cells. EMBO J 26:41–52. https://doi.org/10.1038/sj.emboj.7601475.
18. Kardon JR, Vale RD. 2009. Regulators of the cytoplasmic dynein motor. Nat Rev Mol Cell Biol 10:854–865. https://doi.org/10.1038/nrm2804.
19. Vlach J, Lipov J, Rumlova M, Veverka V, Lang J, Srb P, Knejzlik Z, Pichova I, Hunter E, Hrabal R, Ruml T. 2008. D-retrovirus morphogenetic switch driven by the targeting signal accessibility to Tctex-1 of dynein. Proc Natl Acad Sci U S A 105:10565–10570. https://doi.org/10.1073/pnas.0801765105.
20. Caly L, Kassouf VT, Moseley GW, Diefenbach RJ, Cunningham AL, Jans DA. 2016. Fast track, dynein-dependent nuclear targeting of human immunodeficiency virus Vpr protein; impaired trafficking in a clinical isolate. Biochem Biophys Res Commun 470:735–740. https://doi.org/10.1016/j.bbrc.2016.01.051.
21. Desfarges S, Salin B, Calmels C, Andreola ML, Parissi V, Fournier M. 2009. HIV-1 integrase trafficking in S. cerevisiae: a useful model to dissect the

microtubule network involvement of viral protein nuclear import. Yeast 26:39–54. https://doi.org/10.1002/yea.1651.
22. Allan VJ. 2011. Cytoplasmic dynein. Biochem Soc Trans 39:1169–1178. https://doi.org/10.1042/BST0391169.
23. Bader JR, Vaughan KT. 2010. Dynein at the kinetochore: timing, interactions and functions. Semin Cell Dev Biol 21:269–275. https://doi.org/10.1016/j.semcdb.2009.12.015.
24. Cianfrocco MA, DeSantis ME, Leschziner AE, Reck-Peterson SL. 2015. Mechanism and regulation of cytoplasmic dynein. Annu Rev Cell Dev Biol 31:83–108. https://doi.org/10.1146/annurev-cellbio-100814-125438.
25. Dodding MP, Way M. 2011. Coupling viruses to dynein and kinesin-1. EMBO J 30:3527–3539. https://doi.org/10.1038/emboj.2011.283.
26. Martinez-Moreno M, Navarro-Lerida I, Roncal F, Albar JP, Alonso C, Gavilanes F, Rodriguez-Crespo I. 2003. Recognition of novel viral sequences that associate with the dynein light chain LC8 identified through a pepscan technique. FEBS Lett 544:262–267. https://doi.org/10.1016/S0014-5793(03)00516-7.
27. Brault JB, Kudelko M, Vidalain PO, Tangy F, Despres P, Pardigon N. 2011. The interaction of flavivirus M protein with light chain Tctex-1 of human dynein plays a role in late stages of virus replication. Virology 417:369–378. https://doi.org/10.1016/j.virol.2011.06.022.