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Homomeric Interactions between Transmembrane Proteins of Moloney Murine Leukemia Virus

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We have studied homomeric interactions between transmembrane proteins (TM) of the Moloney murine leukemia virus envelope using the *Saccharomyces cerevisiae* two-hybrid system. TM interacts strongly with itself but not with various control proteins. Deletional and mutational analyses indicated that the putative leucine zipper motif in the extracellular domain of TM is essential and sufficient to mediate the binding. The first three repeats of the leucine zipper-like motif are the most important in mediating the interaction. The TM-TM interaction detected in this system may play a role in several stages of viral replication.

Envelope proteins of retroviruses, including those of Moloney murine leukemia virus (Mo-MuLV), are synthesized in infected cells as a glycoprotein precursor (19, 41; for a review, see reference 16). These glycoproteins form oligomers in the lumen of the endoplasmic reticulum and are subsequently cleaved in the Golgi apparatus by host proteases into two subunits: the extracellular surface protein (SU) and the transmembrane protein (TM) which spans the membrane (12, 27). SU and TM remain associated with each other and are anchored to the membrane through TM (32). There are probably multiple contacts in both SU and TM domains responsible for oligomerization (37). Envelope proteins have two major functions during viral replication. First, they mediate binding of the virus to host cells by interacting with specific viral receptors on the host cell surface. This binding of envelope proteins to cellular receptors is mediated by SU (1, 15, 26, 30). Second, they trigger a complex process leading to fusion of viral and cellular membranes. It has been suggested that TM plays an important role in mediating fusion (16, 25, 38).

Sequence analysis indicates that TM proteins of different retroviruses have many structural similarities (14). At the N terminus of TM is a stretch of hydrophobic amino acids that is believed to be directly involved in the fusion of the viral and cellular membranes (17, 36). Another significant feature is a leucine zipper-like motif (21) located next to the C terminus of the fusion peptide (10, 37). There follows a cluster of several cysteine residues of unknown function and then a second hydrophobic region that is thought to anchor the envelope protein to the viral membrane (34, 41). Finally, there is a cytoplasmic tail whose length varies widely among different viruses. For instance, the cytoplasmic tail of Mo-MuLV TM is short (31 amino acids) and is shortened further by the viral protease, while the cytoplasmic tails of TM of the simian and human immunodeficiency viruses are much longer (about 150 amino acids).

The leucine zipper structure was first described in transcription factors such as c-*fos* and c-*jun* (21). It contains heptad repeats of leucine residues beginning at the *i*th position and, usually, nonpolar amino acids at the $i + 4$ position. Experimental studies showed that the heptad repeats form a coiled-coil structure and mediate the dimerization of these factors (28, 29, 35), which is essential for their functions. For example, the binding of the leucine zippers in c-*fos* and c-*jun* stabilizes a structure formed by adjacent basic regions required for DNA binding activity; mutations that disrupt the dimerization abolish DNA binding functions (20). Dimerization is thought to be mediated by a hydrophobic surface formed by leucine residues in the *i* positions and hydrophobic residues in the $i + 4$ positions of the heptad repeats. It has become apparent that leucine zipper motifs are present in many other proteins which are not involved in transcription (5). Leucine zipper-like motifs found in viral TM proteins, unlike the classical leucine zipper motifs, do not always contain leucine in the *i* positions of heptad repeats. In Mo-MuLV TM, for instance, there is an asparagine instead of a leucine in the third *i* position of the heptad repeat (10). However, recent studies indicated that these altered heptad repeats are still competent to form a coiled-coil structure. For example, a peptide that has the same sequence as the heptad repeat found in human immunodeficiency virus TM forms a coiled-coil structure detected by circular dichroism (39). Mutations in an *i* position of the leucine zipper-like motif disrupt the α-helical structure (39, 40). It has been shown that the leucine zipper-like motif in human immunodeficiency virus TM mediates formation of an oligomer of TM (3, 33).

The *Saccharomyces cerevisiae* two-hybrid system has been widely used to study interactions between known proteins and to identify novel proteins by their ability to bind to a known protein as "bait" (9, 13, 24). Two candidate interacting domains can be expressed as fusion proteins, one with a DNA binding domain and one with a transcriptional activation domain; if the proteins interact, a functional transactivator that can induce expression of an appropriate indicator gene is formed. Because transmembrane and secreted proteins exist in an oxidizing environment outside the cell, such proteins might be considered unlikely to be able to fold properly, interact, and activate reporter genes in the yeast nucleus (24). Nevertheless, we tested whether the system could detect interactions between TM proteins of Mo-MuLV. DNA segments encoding the whole TM as well as various portions of TM were amplified by PCR with synthetic oligonucleotides as primers and cloned into yeast vectors to direct the synthesis of fusion proteins (9, 13). Viral proteins (Fig. 1A) were fused to the C terminus of the DNA binding domain of transcription factors Gal4 (in plasmid pMA424) and LexA (pSH2-1) or the activation do-

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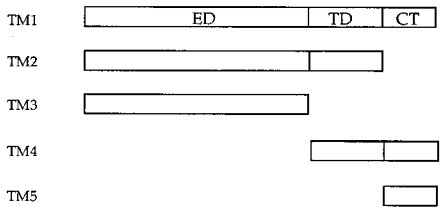
A



B

BD / AD	LexA					Gal4		
	TM1	TM2	TM3	TM4	TM5	TM1	TM2	TM3
TM1	−	−	+	−	−	+	−	+/-
TM2	−	+/-	+	−	−	+	−	+/-
TM3	−	+/-	++	−	−	+	−	++
TM4	−	−	+/-	−	−	−	−	−
TM5	−	−	+/-	−	−	−	−	−

FIG. 1. Interactions between different segments of TM. (A) Schematic diagram of the envelope TM protein and segments of TM included either in LexA or Gal4 DNA binding domain fusions or in Gal4 activation domain fusions. ED, extracellular domain of TM; TD, transmembrane domain; CT, cytoplasmic tail. (B) Interactions between different constructs were examined by the yeast two-hybrid system. The level of *lacZ* expression in the appropriate host was determined by lifting transformants onto nitrocellulose filters and staining them with X-Gal for 4 h at 30°C. ++, stained dark blue; +, stained medium blue; +/−, stained light blue; −, white; AD, activation domain; BD, binding domain.

main of Gal4 (pGADNOT) (18, 23). Possible interactions were tested by transforming yeast strains with pairs of plasmids which encode DNA-binding fusion proteins and activation fusion proteins (Fig. 1B) and then scoring for expression of β-galactosidase produced from the reporter gene by X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) stain of colony replicas on nitrocellulose. LexABD-TM3, containing the extracellular portion of TM, interacted strongly with full-length TM and fragments containing the extracellular portion of TM in *S. cerevisiae* CTY10-5d. In the standard β-galactosidase assays, cotransformants of TM3 constructs turned blue with X-Gal in 30 min at 30°C, suggesting strong interactions between these proteins. LexABD-TM2, containing the extracellular and transmembrane domains of TM, interacted weakly with Gal4AD-TM2 and -TM3. The full-length fusion protein LexABD-TM1 did not interact with any partner, suggesting that this protein was unstable or could not fold properly. Constructs LexABD-TM4 and LexABD-TM5, lacking the extracellular domain, also failed to interact.

To demonstrate specificities of the TM-TM interaction, various tests were performed with TM3 constructs (Table 1). Plasmids encoding fusion proteins LexABD-TM3 and Gal4AD-TM3 introduced into *S. cerevisiae* individually did not activate expression of β-galactosidase. Similarly, plasmids encoding LexABD-TM3 together with vectors encoding the Gal4 activation domain alone (Gal4AD) did not activate.

We extended these results using a second *S. cerevisiae* strain, GGY1::171 (9, 18, 23). In this set of experiments, a Gal4BD-TM1 fusion protein containing the full-length TM interacted with Gal4AD-TM1, -TM2, or -TM3 fusion proteins but not with -TM4 and -TM5 fusions (Fig. 1B). Gal4BD-TM3 also interacted strongly with Gal4AD-TM3 and weakly with Gal4AD-TM1 and Gal4AD-TM2 but did not interact with Gal4AD-TM4 and Gal4AD-TM5 (Fig. 1B). The Gal4BD-TM2 fusion protein exhibited no binding activity to all five Gal4DB fusion proteins examined, probably because of instability or misfolding. As for the LexA fusions, expression of any of the Gal4-TM fusions alone, or with a partner lacking the TM extracellular domain, did not activate β-galactosidase expression (Table 1). Thus, the TM3-TM3 interaction was always dependent on the presence of both TM3 constructs and was observed with two different hosts, indicating that the interaction is independent of yeast strains. These results show that TM can mediate homomeric interactions and suggest that the binding site resides in the extracellular domain of TM.

To determine whether TM3 interacts with other retroviral proteins, CTY10-5d was cotransformed with the LexABD-TM3 fusion construct and with control plasmids encoding Gal4AD fusions of *gag*, integrase, and reverse transcriptase of Mo-MuLV. No blue color was detected in the X-Gal assays of these cotransformants, indicating that LexABD-TM3 is not capable of interacting with these proteins in this system. Interactions of Gal4BD-TM3 with the same fusion constructs were also examined with the GGY1::171 strain. The results similarly showed that Gal4BD-TM3 did not interact with fusions containing *gag*, integrase, or reverse transcriptase of Mo-MuLV. A fusion protein containing the large T antigen of simian virus 40 was also examined; no interaction between T antigen and TM was detected (Table 1). The results indicate that the homomeric interaction between TM proteins is specific.

The primary sequence of TM3 contains a potential leucine zipper motif, but it is not clear whether the motif is the essential feature for the interaction. To map the exact region that is important in mediating the homomeric interactions, deletion mutants were prepared by using synthetic oligonucleotides as primers and PCR and examined by the two-hybrid system (Fig. 2). Deletion mutants TMd1 to TMd6 and TMd8, which retain the leucine zipper-like motif, were capable of interacting with either the wild-type fusion protein or themselves. The observation that TMd4, which contains only the leucine zipper-like motif, was active suggests that the motif is sufficient to mediate the interaction (Fig. 2). These different deletion mutants also interacted with each other (data not shown). However, two deletion mutants that contain only the N-terminal region or the C-terminal region of the extracellular domain of TM and lack the leucine zipper-like motif (TMd9 and TMd10) com-

TABLE 1. Specificity of TM-TM interactions[a]

Fusion protein	Operator	β-Galactosidase activity[b]
LexABD-TM3 + GAL4AD-TM3	*lexA*	++
LexABD-TM3 + GAL4AD	*lexA*	−
LexABD-TM3 + GAL4AD-*gag*	*lexA*	−
LexABD-TM3 + GAL4AD-IN	*lexA*	−
LexABD-TM3 + GAL4AD-RT	*lexA*	−
LexABD-TM3	*lexA*	−
GAL4AD-TM3	*lexA*	−
GAL4BD-TM3 + GAL4AD-TM3	UAS_G	++
GAL4BD-TM3 + GAL4AD	UAS_G	−
GAL4BD-TM3 + GAL4AD-*gag*	UAS_G	−
GAL4BD-TM3 + GAL4AD-RT	UAS_G	−
GAL4BD-TM3 + GAL4AD-T antigen	UAS_G	−
GAL4BD + GAL4AD-TM3	UAS_G	−
GAL4BD-TM3	UAS_G	−
GAL4AD-TM3	UAS_G	−

[a] The promoter in each interaction was *GAL1*.
[b] ++, stained dark blue; −, white with X-Gal stain.

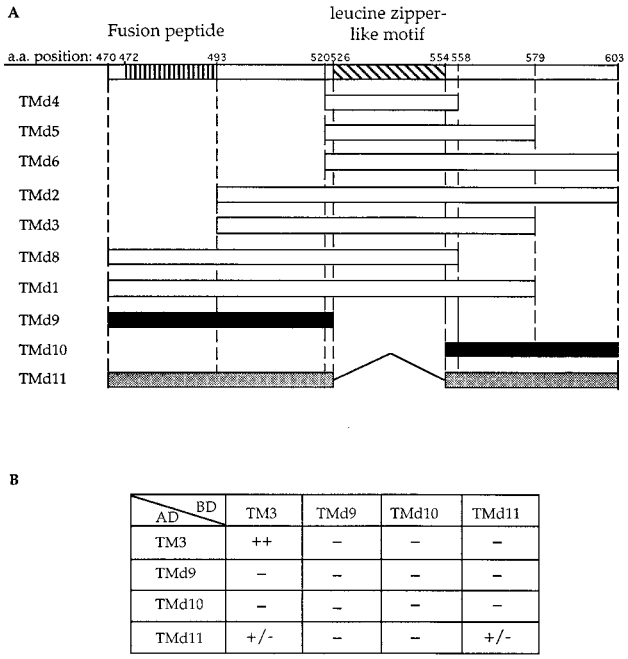


FIG. 2. Effects of deletions in the extracellular domain of the envelope TM protein on TM-TM interaction. (A) Schematic diagram showing the extracellular domain of TM, with the fusion peptide and the leucine zipper-like motif indicated. Regions retained in the various deletion mutants are represented with bars. White constructs indicate retention of binding activity; black constructs indicate loss of activity; shaded constructs indicate significantly decreased activity. Amino acid (a.a.) positions of the borders are indicated relative to the start of $Pr80^{env}$. (B) Interactions between TM3 and three deletion mutants, TMd9, TMd10, and TMd11, were tested in all possible pairings by the two-hybrid system. Symbols and abbreviations are as described in the legend to Fig. 1.

pletely lost the ability to interact with TM3 or themselves (Fig. 2B). A third deletion mutant in which the leucine zipper-like motif was replaced by a few amino acids (VDRIL) generated during construction of the mutant showed a low level of binding activity (Fig. 2). Thus, there may be weak binding sites in the flanking regions. The results indicate that the leucine zipper-like motif is essential and sufficient to mediate the strong homomeric interaction of TM.

To further investigate the role of the leucine zipper-like motif in the homomeric interaction of TM, a series of substitution mutations was introduced into the extracellular domain of TM. Two strategies were employed in constructing these mutants. First, we took advantage of existing mutants (2) and used PCR to transfer the extracellular domains containing mutations into the yeast plasmids. These mutations, named with Arabic numbers (Tables 1 and 2), are scattered throughout the extracellular domain. The second approach involved site-specific mutagenesis by standard methods (22, 31) to make new substitutions within the leucine zipper-like repeat (Fig. 3). The mutants were then tested for activation of β-galactosidase in concert with a wild-type TM3 partner. The results indicate that mutants with alterations in the fusion peptide, in positions other than *i* and *i* + 4 of the leucine zipper-like motif, and in other regions of the extracellular domain of TM all showed wild-type binding activity (Table 2). All mutations introduced in the first and second leucine positions of the leucine zipper-like motif abolished or significantly decreased the binding activity of these mutants (Table 2). It appears that the four *i* positions are not equivalent with regard to their susceptibility to mutagenesis. An asparagine-to-aspartic acid change in the third *i* position seemed not to affect binding very much, but an asparagine-to-proline mutation, on the other hand, significantly disrupted the homomeric interaction. A change from leucine to lysine in the fourth *i* position had no effect on the binding activity of TM (Table 2). These results confirm the importance of leucines within the leucine zipper-like motif and support the notion that the motif mediates the homomeric interaction of TM. The first, second, and possibly third repeats seem most important for dimerization.

TABLE 2. Effects of substitution mutations on the TM-TM interaction

Mutant	Amino acid change(s)	TM-TM interaction (β-galactosidase activity)[a]
56	G-482→R	++
58	G-493→R	++
55	E-470→K	++
26	E-470→K, A-490→T	++
63	D-515→N, G-543→S, R-573→K	++
11	G-552→R, C-562→Y	++
61	R-541→Q, G-553→E, C-555→Y, E-561→K	++
TML1-1	L-526→D	–
TML1-2	L-526→A	–
TML1-3	L-526→P	–
TML2-1	L-533→K	+/–
TML2-2	L-533→A	–
TML2-3	L-533→P	–
TMN3-1	N-540→D	+
TMN3-2	N-540→P	+/–
TML4-1	L-547→K	++

[a] ++, stained dark blue; +, stained blue; +/–, stained light blue; –, white with X-Gal stain.

In conclusion, we have demonstrated a homomeric interaction between the TM proteins of Mo-MuLV using the yeast two-hybrid system. The results suggest that TM may form dimers or multimers and that the binding site is located in the outer portion of TM. Studies of deletion mutants of TM have further located the binding site to the leucine zipper-like motif, predicted from analyses of amino acid sequences (10, 14, 37). Our data suggest that the leucine zipper-like motif is sufficient to mediate the homomeric interaction. Mutations altering the fusion peptide and other regions of TM did not affect the homomeric interaction, and changes only of the leucines to other amino acids within the leucine heptad repeat abolished

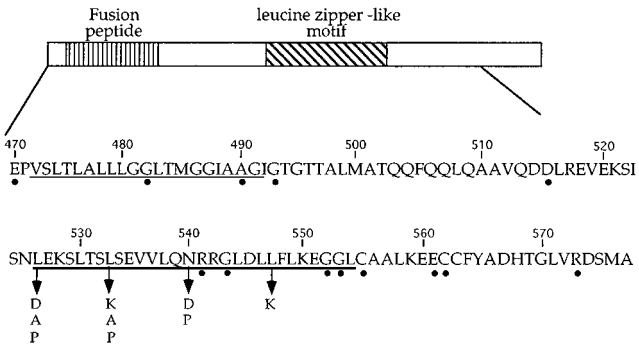


FIG. 3. Mutagenized region of the Mo-MuLV envelope TM. Residues altered in mutants described previously (2) are indicated with black dots. Newly generated single substitution mutations within the leucine zipper-like motif are shown below the arrows. The fusion peptide and the leucine zipper-like motif are underlined.

or decreased the ability of TM proteins to interact with each other, indicating a critical role of leucines in mediating the homomeric interaction. We cannot simply correlate these phenotypes with the behavior of envelopes carrying these mutations in the intact virus; even those mutants which dimerized normally were replication defective (2), suggesting that other *env* functions are disrupted by many of these mutations.

It is not clear at what time in the viral life cycle the interaction is used by the viral envelope proteins. Although it was predicted that the leucine zipper-like region was involved in the oligomerization of envelope proteins (10, 14), recent studies point to a role of the motif in the fusion of viral and cellular membranes. Mutations within the leucine zipper-like motif of human immunodeficiency virus type 1 did not disrupt viral envelope oligomerization but rather affected the fusion process during viral entry into the cell (8, 11, 39). Similarly, a leucine zipper motif was identified in the fusion (F) protein of measles virus and shown to play an essential role in fusion and infectivity (4). It was proposed that during fusion, the lateral movement of individual viral glycoproteins leads to the formation of a higher-order fusogenic structure that is required for the penetration of the fusion peptide into the cellular membrane (25, 38). In addition, through structure determination of influenza virus hemagglutinin before and after acidification, large movements of protein domains that are thought to mimic changes during fusion have been detected (6, 7). Although there is no detailed model as to how TM mediates fusion, it is possible that rearrangements of TM bring multiple fusion peptides together to form fusogenic structures. Features of TM are consistent with this hypothesis. Our results suggest that within the leucine zipper-like motif, the leucines closer to the fusion peptide are more critical with respect to homomeric interaction. Thus, it will be interesting to examine whether mutations in the first two *i* positions have a more profound effect on fusion in vivo.

Finally, we note that the interactions scored here represent an extension of the yeast two-hybrid system to proteins that are normally extracellular. It is likely that a subset of the interactions between extracellular domains of other proteins can be similarly monitored and characterized by the system, as long as posttranslational modifications are not required for binding.

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REFERENCES

1. **Battini, J., J. M. Heard, and O. Danos.** 1992. Receptor choice determinants in the envelope glycoproteins of amphotropic, xenotropic, and polytropic murine leukemia viruses. J. Virol. **66:**1468–1475.
2. **Berkowitz, R. D., and S. P. Goff.** 1993. Point mutations in Moloney murine leukemia virus envelope protein: effects on infectivity, virion association, and superinfection resistance. Virology **196:**748–757.
3. **Bernstein, H. B., S. P. Tucker, S. R. Kar, S. A. McPherson, D. T. McPherson, J. W. Dubay, J. Lebowitz, R. W. Compans, and E. Hunter.** 1995. Oligomerization of the hydrophobic heptad repeat of gp41. J. Virol. **69:**2745–2750.
4. **Buckland, R., E. Malvoisin, P. Beauverger, and F. Wild.** 1992. A leucine zipper structure present in the measles virus fusion protein is not required for its tetramerization but is essential for fusion. J. Virol. **73:**1703–1707.
5. **Buckland, R., and F. Wild.** 1989. Leucine zipper motif extends. Nature (London) **338:**547.
6. **Bullough, P. A., F. M. Hughson, J. J. Skehel, and D. C. Wiley.** 1994. Structure of influenza haemagglutinin at the pH of membrane fusion. Nature (London) **371:**37–43.
7. **Carr, C. M., and P. S. Kim.** 1993. A spring-loaded mechanism for the conformational change of influenza hemagglutinin. Cell **73:**823–832.
8. **Chen, S. S.-L., C. Lee, W. Lee, K. McIntosh, and T.-H. Lee.** 1993. Mutational analysis of the leucine zipper-like motif of the human immunodeficiency virus type 1 envelope transmembrane glycoprotein. J. Virol. **67:**3615–3619.
9. **Chien, C.-T., P. L. Bartel, R. Sternblanz, and S. Fields.** 1991. The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:**9578–9582.
10. **Delwart, R. L., and G. Mosialos.** 1990. Retroviral envelope glycoproteins contain a leucine zipper like repeat. AIDS Res. Hum. Retroviruses **6:**703–706.
11. **Dubay, J. W., S. J. Roberts, B. Brody, and E. Hunter.** 1992. Mutations in the leucine zipper of the human immunodeficiency virus type 1 transmembrane glycoprotein affect fusion and infectivity. J. Virol. **66:**4748–4756.
12. **Famulari, N. G., D. L. Buchhagen, H.-D. Klenk, and R. Fleissner.** 1976. Presence of murine leukemia virus envelope proteins gp70 and p15(E) in a common polyprotein of infected cells. J. Virol. **20:**501–508.
13. **Fields, S., and O. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature (London) **340:**245–246.
14. **Gallaher, W. R., J. M. Ball, R. F. Garry, M. C. Griffin, and R. C. Montelaro.** 1989. A general model for the transmembrane proteins of HIV and other retroviruses. AIDS Res. Hum. Retroviruses **5:**431–440.
15. **Heard, J. M., and O. Danos.** 1991. An amino-terminal fragment of the Friend murine leukemia virus envelope glycoprotein binds the ecotropic receptor. J. Virol. **65:**4026–4032.
16. **Hunter, E., and R. Swanstrom.** 1990. Retrovirus envelope glycoproteins. Curr. Top. Microbiol. Immunol. **157:**187–253.
17. **Jones, J. S., and R. Risser.** 1993. Cell fusion induced by the murine leukemia virus envelope glycoprotein. J. Virol. **67:**67–74.
18. **Kalpana, G. V., and S. P. Goff.** 1993. Genetic analysis of homomeric interactions of human immunodeficiency virus type 1 integrase using the yeast two-hybrid system. Proc. Natl. Acad. Sci. USA **90:**10593–10597.
19. **Karshin, W. L., L. J. Arcement, R. B. Naso, and R. B. Arlinghaus.** 1977. Common precursor for Rauscher leukemia virus gp69/71, p15(E), and p12(E). J. Virol. **23:**787–798.
20. **Kouzarides, T., and E. Ziff.** 1988. The role of the leucine zipper in the fos-jun interaction. Nature (London) **336:**646–651.
21. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1988. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240:**1759–1764.
22. **Landt, O., H.-P. Grunert, and U. Hahn.** 1990. A general method for rapid site-directed mutagenesis using the polymerase chain reaction. Gene **96:**125–128.
23. **Luban, J., K. L. Bossolt, E. K. Franke, G. V. Kalpana, and S. P. Goff.** 1993. Human immunodeficiency virus type 1 gag protein binds to cyclophilins A and B. Cell **73:**1067–1078.
24. **Luban, J., and S. P. Goff.** 1995. The yeast two-hybrid system for studying protein-protein interactions. Curr. Opin. Biotechnol. **6:**59–64.
25. **Marsh, M., and A. Helenius.** 1989. Viral entry into animal cells. Adv. Virus Res. **36:**107–151.
26. **Morgan, R. A., O. Nussbaum, D. D. Muenchau, L. Shu, L. Couture, and W. F. Anderson.** 1993. Analysis of the functional and host range-determining regions of the murine ecotropic and amphotropic retrovirus envelope proteins. J. Virol. **67:**4712–4721.
27. **Ng, V. L., T. G. Wood, and R. B. Arlinghaus.** 1982. Processing of the env gene products of Moloney murine leukemia virus. J. Gen. Virol. **59:**329–343.
28. **O'Shea, E., R. Rutkowski, and P. S. Kim.** 1989. Evidence that the leucine zipper is a coiled-coil. Science **243:**538–542.
29. **O'Shea, E. K., J. K. Klemm, P. S. Kim, and T. Alber.** 1991. X-ray structure of the GCN4 leucine zipper, a two stranded, parallel coiled coil. Science **254:**539–544.
30. **Ott, D., and A. Rein.** 1992. Basis for receptor specificity of nonecotropic murine leukemia virus surface glycoprotein $gp70^{SU}$. J. Virol. **66:**4632–4638.
31. **Perrin, S., and G. Gilliland.** 1990. Site-specific mutagenesis using asymmetric polymerase chain reaction and a simple mutant primer. Nucleic Acids Res. **18:**7433–7438.
32. **Pinter, A., and W. J. Honnen.** 1983. Topography of murine leukemia virus envelope proteins: characterization of transmembrane components. J. Virol. **46:**1056–1060.
33. **Poumbourios, P., W. El Ahmar, D. A. McPhee, and B. E. Kemp.** 1995. Determinants of human immunodeficiency virus type 1 envelope glycoprotein oligomeric structure. J. Virol. **69:**1209–1218.
34. **Ragheb, J. A., and W. F. Anderson.** 1994. Uncoupled expression of Moloney murine leukemia virus envelope polypeptides SU and TM: a functional analysis of the role of TM domains in viral entry. J. Virol. **68:**3207–3219.
35. **Rasmussen, L. J., D. Benvegnu, E. K. O'Shea, P. S. Kim, and T. Abler.** 1991. X-ray scattering indicates that the leucine zipper is a coiled coil. Proc. Natl. Acad. Sci. USA **88:**561–564.
36. **Stegmann, T., R. W. Doms, and A. Helenius.** 1989. Protein-mediated membrane fusion. Annu. Rev. Biophys. Biophys. Chem. **18:**187–211.
37. **Tucker, S. P., R. Sprinvas, and R. W. Compans.** 1991. Molecular domains involved in oligomerization of the Friend murine leukemia virus envelope glycoprotein. Virology **185:**710–720.

38. **White, J.** 1992. Membrane fusion. Science **258:**917–924.
39. **Wild, C., J. W. Bubay, T. Greenwell, T. Baird, Jr., T. G. Oas, C. McDanal, E. Hunter, and T. Matthews.** 1994. Propensity for a leucine zipper-like domain of human immunodeficiency virus type 1 gp41 to form oligomers correlates with a role in virus-induced fusion rather than assembly of the glycoprotein complex. Proc. Natl. Acad. Sci. USA **91:**12676–12680.
40. **Wild, C., T. Oas, C. McDanal, D. Bolognesi, and T. Matthews.** 1992. A synthetic peptide inhibitor of human immunodeficiency virus replication: correlation between solution structure and viral inhibition. Proc. Natl. Acad. Sci. USA **89:**10537–10541.
41. **Witte, O. N., and D. F. Wirth.** 1979. Structure of the murine leukemia virus envelope glycoprotein precursor. J. Virol. **29:**735–743.