Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Integrins as virus receptors

Adenovirus uses a receptor to attach to host cells and a second receptor, an integrin, for internalization. As in cell–cell contact, more than one receptor may contribute to a productive interaction.

Adenovirus, which causes respiratory and gastrointestinal infections, has been found to use a host cell adhesion molecule, an integrin, as a receptor [1]. At first glance, this finding is not surprising. Well-known for their role in cell–extracellular matrix and cell–cell adhesion [2], integrins have already been identified [3] or implicated [4,5] as receptors for three picornaviruses (Table 1). Moreover, an expanding repertoire suggests that virtually any cell-surface molecule can function as a virus receptor. What is interesting is that, rather than being used as a primary attachment receptor for adenovirus, the integrin in this case seems to be playing a crucial role in a post-binding step in virus entry into the host cell [1].

There have been hints previously in the literature (cited in [1]) that an integrin might be involved in adenovirus entry. First, the penton base, a component of the virus capsid (Fig. 1), contains five copies of polypeptide III, a protein that bears the integrin-binding sequence motif RGD (Arg–Gly–Asp). Poised at each of the 12 icosohedral vertices of the virus particle (Fig. 1a), the penton bases provide 12 potential multivalent attachment sites for integrins. Second, soluble penton base protein detaches cells from their substratum, consistent with a function for the penton base as an integrin ligand. And finally, adenovirus internalization requires divalent cations, which are also required for the functional integrity of integrin αβ heterodimers.

The recent study by Wickham *et al.* [1] shows that penton base protein is a ligand for integrins comprised of $\alpha_v\beta_3$ and /or $\alpha_v\beta_5$ chains, and that the penton base–integrin interaction is important for virus infection. In this study, cells expressing α_v integrins (α_v-positive cells) attached and spread on dishes coated with purified penton base protein. Cell–penton base attachment was blocked by the RGD-containing peptide GRGDSP, and by monoclonal antibodies against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. In addition, α_v-positive cells were more infectable than their α_v-negative counterparts, and infection could be inhibited by GRGDSP peptide, penton base protein or $\alpha_v\beta_3/\alpha_v\beta_5$ monoclonal antibodies.

Although the penton base–integrin interaction is required for virus infection, it is not needed for the virus to bind to host cells [1]. Instead, the primary interaction between virus and cell employs the virus fiber (cited in [1,6]), a stalk with a distal cell-binding knob, which projects from the center of each penton base (Fig. 1b). The fiber binds to a distinct cell-surface receptor: although not molecularly characterized, there are approximately 16 000 receptors per HeLa cell that bind to the fiber with nanomolar affinity, whereas the interaction between the penton base

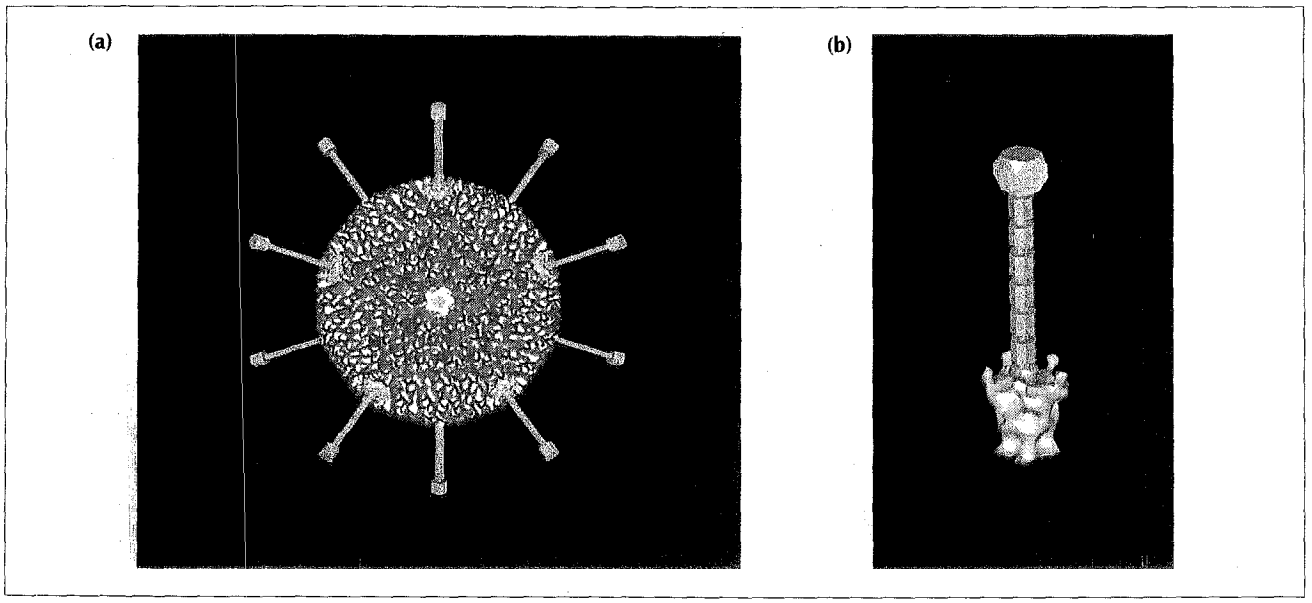

Fig. 1. Three-dimensional reconstructions of the major components of the adenovirus capsid **(a)** and penton complex **(b)** with the full fibre length modelled. Each penton complex is made up of one penton base (yellow) plus one protruding fiber (green); hexons are shown in blue. The trimeric fiber projects further from the penton complex than revealed by image reconstruction (R Burnett and P Stewart, personal communication): the additional projection (~300 Å) is modelled in green. The five protrusions on each penton base might be the sites of interaction with the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins. (Photographs courtesy of Phoebe Stewart and Roger Burnett).

Table 1. Examples of cell surface molecules used as virus receptors.

Cell surface molecule	Virus	Family	References
IgG superfamily members			
ICAM-1	Rhinovirus	Picornaviridae	[17]
Poliovirus receptor	Poliovirus	Picornaviridae	[17]
CD4	HIV-1, HIV-2, SIV	Retroviridae	[13,17]
Carcinoembryonal antigen	Mouse Hepatitis virus	Coronaviridae	[18]
Multimembrane spanning transporters			
(basic amino acid) transporter	Murine Leukemia virus (ecotropic)	Retroviridae	[13,17]
(? phosphate) permease	Gibbon Ape Leukemia virus	Retroviridae	[13,17]
permease*	Feline Leukemia virus, subgroup B	Retroviridae	[13]
permease*	Murine Leukemia virus (amphotropic)	Retroviridae	Footnote*
Growth factor (related) receptors			
Low Density Lipoprotein (related) receptor	Rous Sarcoma virus, subgroup A	Retroviridae	[19]
Erythropoietin receptor†	Murine Leukemia virus-MCF	Retroviridae	[13]
Integrins			
$\alpha_2\beta_1$	Echovirus	Picornaviridae	[3]
$\alpha_x\beta_y$‡	Foot and Mouth Disease virus	Picornaviridae	[4]
$\alpha_x\beta_y$‡	Coxsackie virus	Picornaviridae	[5]
$\alpha_v\beta_3/\beta_5$	Adenovirus	Adenoviridae	[1]
Other proteins			
Complement Receptor (CR2)	Epstein-Barr Virus	Herpesviridae	[17]
Aminopeptidase N	Human Coronavirus 229E	Coronaviridae	[20]
67 kD non-integrin laminin receptor§	Sindbis virus	Togaviridae	[21]
Carbohydrates and lipids			
Sialic acid-containing glycoproteins and glycolipids[IP]	Influenza virus	Orthomyxoviridae	[17]
	Sendai virus	Paramyxoviridae	[17]
Heparin sulfate	Herpes Simplex virus	Herpesviridae	[11]
Galactosylceramide#	HIV	Retroviridae	[13,17]

The $\alpha_v\beta_3/\beta_5$ integrin is the only cell surface molecule listed that is known to serve as a secondary virus receptor. It is likely that other (non-integrin) secondary virus receptors will be identified. Some primary virus receptors may also play secondary role(s) in post-binding steps of virus entry.

* The receptor for Feline Leukemia virus, subgroup B has been implicated based on cross interference with the receptor for Gibbon Ape Leukemia virus. The amphotropic murine leukemia virus receptor is ~60 % identical to the Gibbon Ape Leukemia virus receptor (B O'Hara and J Cunningham, personal communication).

† The Murine leukemia virus-MCF envelope glycoprotein binds to the erythropoietin receptor. It has not yet been formally shown that the erythropoietin receptor is required for virus binding or entry.

‡ Binding of Foot and Mouth Disease virus and Coxsackie virus to cells is inhibited by RGD-containing peptides, implicating integrins as their receptors. The notation $\alpha_x\beta_y$ is used, as the identity of the (presumed) integrins involved is not known.

§ The 67 kD non-integrin laminin receptor has been implicated as a Sindbis virus receptor on mammalian cells. Sindbis virus appears to use a different receptor on avian cells.

IP All orthomyxoviruses and paramyxoviruses appear to use sialic acid-containing structures as receptors.

\# Galactosylceramide has been implicated as an 'alternative' receptor [13] in $CD4^-$ human cells. There is no evidence for it being the secondary receptor/factor (see text) required for infection of $CD4^+$ human cells.

and $\alpha_v\beta_3/\alpha_v\beta_5$ integrins has different characteristics — a K_d of 60 nM and 90 000 interaction sites per HeLa cell. Interestingly, the complex of penton base and protruding fiber (Fig. 1b) binds to cells with the same characteristics as the isolated fiber [1].

The findings that virus internalization is enhanced (as assessed by a protease-resistance assay) in α_v-positive cells relative to α_v-negative cells, and that internalization is inhibited by treatments that block $\alpha_v\beta_3/\alpha_v\beta_5$ integrin function, led Nemerow and colleagues [1] to conclude that the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins are required for virus internalization. They therefore denoted these integrins as 'virus internalization receptors', as distinct from the known 'virus attachment receptors'. The penton base–integrin interaction is further implicated in virus internalization by the observation that, whereas purified penton bases are readily internalized by α_v-positive cells, purified fibers are not. The role of integrins in adenovirus internalization [1] mirrors their role in the internalization of invasive bacteria [7,8].

Primary and secondary virus receptors

The demonstration that $\alpha_v\beta_3/\alpha_v\beta_5$ integrins bind specifically to a component of the adenovirus capsid and that they are involved in a post-binding step of adenovirus entry into host cells indicates that the integrins are not being used as primary virus receptors but rather as secondary receptors. Secondary virus receptors might be required

for internalization (endocytosis; see Fig. 2b), as are the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins for adenovirus infection [1], and/or they may be involved in genome penetration — delivery of the viral genome, with or without associated capsid, into the host cell cytoplasm (Fig. 2d). The existence of secondary virus receptors echoes a theme from the field of cell adhesion: multiple ligand–receptor pairs are often needed to generate a productive interaction.

Many cells require two or more ligand–receptor interactions to foster specific and tight adhesion or to signal the consequences of the cell–cell or cell–matrix interaction [2]. For example, syndecan-1, a heparin sulfate proteoglycan, functions as a 'co-receptor' with a β_1 integrin in binding fibronectin, and with the basic growth factor receptor in binding growth factor [9]. For an inflammatory reaction, a cascade of ligand–receptor pairs involving selectins and selectin receptors followed by integrins and their receptors — in this case the IgG superfamily member ICAM-1 — is required for leukocytes to cross the endothelial wall lining blood vessels and enter the tissues [10]. Even more complex are the multiple interactions involved in the binding and subsequent activation of T cells by antigen-presenting cells [2].

With the numerous precedents from cell–cell interactions [2,8,9], it is not all that surprising that secondary receptors have surfaced in the virus world. Herpes simplex virus (HSV) uses host cell-surface heparin sulfate proteoglycans as attachment receptors [11]. A second, saturable — but yet to be identified — heparin sulfate-independent cell-surface receptor has been implicated in a post-binding stage of HSV entry [12]. Although the human immunodeficiency virus (HIV) uses the lymphocyte cell surface molecule CD4 as its major receptor, CD4 seems to be necessary but not sufficient for HIV fusion and infection of cells. HIV can bind to murine cells expressing human CD4 but the virus will neither fuse with the plasma membrane nor infect such cells, even though the HIV genome is infectious when introduced artificially into murine cells. Although a modification may occur to CD4 in human cells that does not occur in murine cells; the search for a secondary receptor/factor required for HIV entry into cells is ongoing [13].

So, adenovirus, HSV, and perhaps HIV, use secondary virus receptors — cell-surface receptors distinct from their primary attachment receptors — to enter cells. As these examples include enveloped and non-enveloped viruses, RNA and DNA viruses, and viruses that infect cells at neutral pH (cell surface) or low pH (endosomes), secondary virus receptors may be more broadly used by viruses than is currently recognized. As virus receptors, members of the integrin family are noteworthy for interrelated reasons: they include the first molecularly identified secondary virus receptors ($\alpha_v\beta_3/\alpha_v\beta_5$), which are also the first secondary virus receptors for which a role has been clarified, and they are used by different viruses as either primary [3] or secondary [1] virus receptors.

The role of integrins in adenovirus infection

Dissection of the pathway of adenovirus entry into cells reveals several points at which the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins could be involved (Fig. 2). First of all, the virus binds to the cell surface (Fig. 2a) in a primary interaction between the fiber and its receptor. A second interaction between the penton base and integrins must occur for successful infection [1], but the precise conditions fostering this interaction have yet to be characterized. The penton base receptor might be engaged simultaneously with the fiber, perhaps involving conformational changes in the virus or the receptor, or engaged after discussion of the fiber from the virus particle. Some fibers dissociate early and in a pH-independent fashion during adenovirus infection (U Greber and A Helenius, personal communication).

The second phase of virus entry sees the virus localized to coated pits (Fig. 2b), specialized invaginations of the plasma membrane responsible for receptor-mediated

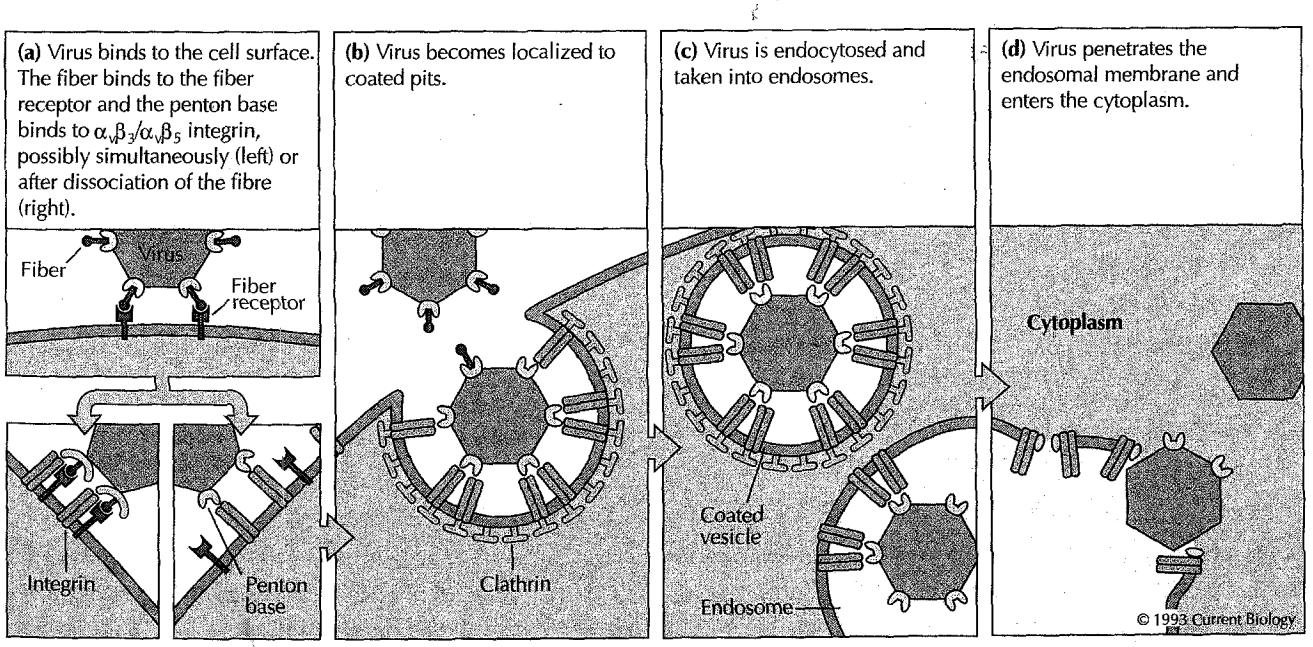

Fig. 2. Pathway of adenovirus entry into cells.

endocytosis. As adenovirus is known to enter cells by receptor-mediated endocytosis [14], the virus must localize to coated pits on binding or shortly thereafter. It is not yet known whether the fiber receptor is a transmembrane protein, much less whether it has a coated-pit localization signal. Nevertheless, the sequences of the cytoplasmic tails of both the β_3 and β_5 integrin chains contain the sequence NPXY found in some coated pit localization signals, although the α_v cytoplasmic tail does not. Some integrins are known to be constitutively endocytosed [15], although β_3 and β_5 have yet to be studied. The fact that five RGD-containing polypeptide III subunits circumscribe each penton base (Fig. 1b) implies that the virus has a built-in mechanism for clustering the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins. Integrin clustering may be a prerequisite for, or may strengthen, coated pit binding.

In the next phase of virus entry, the virus is endocytosed (Fig. 2c). Coated pits pinch off to form coated vesicles that bring the virus to the endosomal compartment. Once localized to coated pits, therefore, the virus may simply 'hitch a ride' into the cell through the normal pathway of receptor-mediated endocytosis. Whether localization to coated pits and/or subsequent stages of endocytosis require the coated pit localization signals of the β_3/β_5 integrins, or — as suggested by Wickham *et al.* [1] — signalling functions of the integrins [2], remains to be determined.

The final phase of virus entry in which integrins might function is when the virus penetrates the endosomal membrane (Fig. 2d). Once inside the endosomal compartment, it is not clear how the adenovirus capsid crosses the endosomal membrane. For enveloped viruses, which have a delimiting lipid bilayer, genome penetration occurs by membrane fusion and involves fusion pores composed of specific viral glycoproteins [16]. If the penton base is involved in penetration of the adenovirus capsid, then there are at least two roles that the $\alpha_v\beta_3/\alpha_v\beta_5$ integrins could play in the process. The penton base–$\alpha_v\beta_3/\alpha_v\beta_5$ interaction might simply bring the virus capsid into close contact with the endosomal membrane (Fig. 2c). In this context, it is interesting that, whereas the penton base binds both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins at neutral pH, it binds only $\alpha_v\beta_5$ and not $\alpha_v\beta_3$ at pH < 5.5 (G Nemerow, personal communication). This finding reflects a previously noted low pH-dependent interaction between penton base and the host cell [1,6], and suggests an especially important role for $\alpha_v\beta_5$ during the endosomal phase of adenovirus entry. In addition to this steric role, the $\alpha_v\beta_5$ integrin may facilitate pore formation and may therefore play an active role in capsid penetration.

In conclusion, adenovirus clearly uses two receptors to enter host cells, a primary receptor for attaching to cells and a secondary receptor, consisting of $\alpha_V\beta_3$ and/or $\alpha_V\beta_5$ integrins for virus internalization. The integrins appear to be required for endocytosis of the virus particle. They may also be required for penetration of the viral genome into the host cell cytoplasm for replication. Future work will clarify precisely how integrins are used as secondary adenovirus receptors and, no doubt, will reveal interesting ways in which other cell surface molecules are used as secondary virus receptors.

References

1. WICKHAM T, MATHIAS P, CHERESH D, NEMEROW G: **Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ promote adenovirus internalization but not virus attachment.** *Cell* 1993, 73:309–319.
2. HYNES, R: **Integrins: versatility, modulation and signalling in cell adhesion.** *Cell* 1992, 69:11–25.
3. BERGELSON J, SHEPLEY KM, CHAN B, HEMLER M, FINBERG R: **Identification of the intergrin VLA-2 as a receptor for echovirus 1.** *Science* 1992, 255:1718–1720.
4. FOX G, PARRY N, BARNETT P, MCGINN B, ROWLANDS D, BROWN F: **The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RGD (arginine–glycine–aspartic acid).** *J Gen Virol* 1989, 70:625–637.
5. RIOVAINEN M, HYYPIA T, PIIRAINEN L, KALKKINEN N, STANWAY G, HOVI T: **RGD-dependent entry of coxsackievirus A9 into host cells and its bypass after cleavage of VP1 protein by intestinal proteases.** *J Virol* 1991, 65:4735–4740.
6. STEWART P, FULLER S, BURNETT R: **Difference imaging of adenovirus: bridging the resolution gap between X-ray cyrstallography and electron microscopy.** *EMBO J* 1993, 12: 2589–2599.
7. ISBERG RR, LOENG JM: **Multiple β1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells.** *Cell* 1990, 6:861–871.
8. RELMAN D, TOUMANEN E, FALKOW S, GOLENBOCK D, SAUKKONEN D, WRIGHT S: **Recognition of a bacterial adhesion by an integrin: Macrophage CR3 ($\alpha_m\beta_2$, CD11b/CD18) binds filamentous hemagglutinin of *Bordetella pertussis*.** *Cell* 1990, 61:1375–1382.
9. BERNFIELD M KOKENYESI R DATO M, HINKES M, SPRING J, GALLO R, LOSE E: **Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans.** In *Annu Rev Cell Biol* 1992, 8 :365–393.
10. LAWRENCE M SPRINGER T: **Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins.** *Cell* 1991, 65:859–873.
11. SPEAR P, SHIEH M-T, HEROLD B, WUDUNN D, KOSHY T: **Heparan sulfate glycosaminoglycans as primary cell surface receptors for herpes simplex virus.** In *Heparin and Related Polysaccharides.* Edited by Lane D, Björk I, Lindahl U. New York: Plenum Press; 1992:341–353.
12. JOHNSON DC, BURKE RL, GREGORY T: **Soluble forms of herpes simplex virus glycoprotein D bind to a limited number of cell surface receptors and inhibit viral entry into cells.** *J Virol* 1990, 64:2569–2576.
13. WEISS R: **Cellular receptors and viral glycoproteins involved in retrovirus entry.** In *The Retroviruses.* Edited by Levy J. New York: Plenum Press; 1992:1–108.
14. VARGA M, WEIBULL C, EVERITT E: **Infectious entry pathway of adenovirus type 2.** *J Virol* 1991, 65:6061–6070.
15. BRETSCHER M: **Circulating integrins: $\alpha_5\beta_1$, $\alpha_6\beta_4$ and Mac-1, but not $\alpha_1\beta_1$, $\alpha_4\beta_1$ or LFA-1.** *EMBO J* 1992, 11:405–410.
16. WHITE JM: **Membrane fusion.** *Science* 1992, 258:917–924.
17. NOMOTO JM: **Cellular receptors for virus infection.** *Semin Virol* 1992, 3:77–133.
18. WILLIAMS R, JIANG G-S, HOLMES K: **Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins.** *Proc Natl Acad Sci USA* 1991, 88:5533–5536.
19. BATES P, YOUNG JAT, VARMUS H: **A receptor for subgroup A Rous sarcoma virus is related to the LDL receptor.** *Cell* 1993, in press.
20. YEAGER C, ASHMUN RA, WILLIAMS RK, CARDELLICHIO CB, SHAPIRO LH, LOOK AT, HOLMES KV: **Human aminopeptidase N is a receptor for human coronavirus 229E.** *Nature* 1992, 357:420–422.
21. WANG K-S, KUHN RJ, STRAUSS EG, OU S, STRAUSS JH: **High-affinity laminin receptor for Sindbis virus in mammalian cells.** *J Virol* 1992, 66:4992–5001.

Judith M. White, Department of Pharmacology and Cell Biology Program, University of California, San Francisco, California 94143-0450, USA.