whereby VASP bound to ActA captures actin filaments, thus allowing assembly of actin monomers at their ends to provide force for motility. VASP/Mena seem to be essential for motility in extracts of mammalian cells, in apparent contradiction to studies of mutants of *Listeria* in which ActA is missing the FPPPP sequences.

It is possible that in these mutant bacteria some VASP can still associate with the actin tails via actin filaments or by binding to other regions or binding partners of ActA[19]. It would be interesting to know where VASP localizes in the permeabilized neutrophils of Weiner *et al.* If the analogy with *Listeria* holds, one might predict that it should be near the tips of the projections and that it might co-localize there with vinculin or zyxin (Fig. 2).

What might the future hold? Although Weiner *et al.* make a strong case for analogy between the two systems (*Listeria* and neutrophils), many questions remain. Does the Arp2/3 complex directly bind to ActA in *Listeria*? Many groups must have attempted to find out, and it is puzzling that there have been no reports of success. The affinity might be weak, or perhaps other factors or post-translational modifications are required to achieve binding. Also, are WASP-family proteins the true cellular analogues of the amino terminus of ActA? If so, how is their localization and activity regulated? Many adaptor proteins bind directly to WASP and to receptor tyrosine kinases, so one possibility is that tyrosine phosphorylation of receptors may regulate the localization or activity of the Arp2/3 complex via adaptor proteins binding to WASP (Fig. 2a). Additionally, because Cdc42 binds directly to WASP and N-WASP, Cdc42 may also have a role in the localization or activation of WASP/N-WASP, possibly via a guanine-nucleotide exchange factor (GEF) or a direct interaction with membrane polyphosphoinositides[5].

As WASP and N-WASP have been associated with Cdc42, which can induce formation of filopodia when activated, and SCAR1 has been implicated in lamellipodia formation and co-immunoprecipitated with the small G protein Rac[10,12], it is tempting to assume that we know all the answers. We could construct models based on parallel pathways such as Rac → SCAR1 → Arp2/3 complex → actin → lamellipodia or Cdc42 →WASP/N-WASP→Arp2/3 complex→actin→filopodia. But while there may be some truth in these ideas, it seems highly unlikely that any simple linear pathway could lead to such complex structures as lamellipodia and filopodia. In addition to the Arp2/3 complex, many cytoskeletal proteins (such as gelsolin, profilin, capping protein and cofilin, to name but a few), kinases and adaptor proteins are connected with the activation of Rac and Cdc42 through cell-surface receptors.

The role of VASP/Mena and zyxin will need to be further defined, as they are important in *Listeria* motility but their role in mammalian cells is less clear. Mena seems to be important for cell motility in developing neurons and localizes to the tips of growth-cone filopodia[20]. Castellano *et al.* found that when activated Cdc42 was clustered at the plasma membrane, protrusions resembling *Listeria* actin tails formed which contained VASP and zyxin, but apparently not the Arp2/3 complex[21]. These results suggest that VASP/Mena proteins can perhaps promote actin assembly independently of Arp2/3 complex. Clustering of WASP at the plasma membrane had a similar but less dramatic effect, indicating that there are likely to be multiple pathways leading from Cdc42 to cytoskeletal reorganization[21]. Understanding how these pathways are coordinated to allow cells to respond to signals with changes in shape and motility is likely to keep us busy for a long time. □

Laura M. Machesky is in the Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, UK.
e-mail: l.m.machesky@bham.ac.uk

1. Dramsi, S. & Cossart, P. *Annu. Rev. Cell Dev. Biol.* **14,** 137–166 (1998).
2. Weiner, O. D. *et al. Nature Cell Biol.* **1,** 75–81 (1999).
3. Bailly, M. *et al. J. Cell Biol.* **145,** 331–345 (1999).
4. Machesky, L. M. *et al. Proc. Natl Acad. Sci. USA* **96,** 3739–3744 (1999).
5. Rohatgi, R. *et al. Cell* **97,** 221–231 (1999).
6. Winter, D., Lechler, T. & Li, R. *Curr. Biol.* **9,** 501–504 (1999).
7. Welch, M. D., Iwamatsu, A. & Mitchison, T. J. *Nature* **385,** 265–269 (1997).
8. Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. A. & Mitchison, T. J. *Science* **281,** 105–108 (1998).
9. Mullins, R. D., Heuser, J. A. & Pollard, T. D. *Proc. Natl Acad. Sci.USA* **95,** 6181–6186 (1998).
10. Machesky, L. M. & Insall, R. H. *Curr. Biol.* **8,** 1347–1356 (1998).
11. Bear, J. E., Rawles, J. F. & Saxe III, C. L. *J. Cell Biol.* **142,** 1325–1335 (1998).
12. Miki, H., Suetsugu, S. & Takenawa, T. *EMBO J.* **17,** 6932–6941 (1998).
13. Ma, L., Rohatgi, R. & Kirschner, M. W. *Proc. Natl Acad. Sci. USA* **95,** 15362–15367 (1998).
14. Ma, L., Cantley, L. C., Janmey, P. A. & Kirschner, M. W. *J. Cell Biol.* **140,** 1125–1136 (1998).
15. Moreau, V. & Way, M. *FEBS Lett.* **427,** 353–356 (1998).
16. Niebuhr, K. *et al. EMBO J.* **16,** 5433–5444 (1997).
17. Gertler, F. B., Niebuhr, K., Reinhard, M., Wehland, J. & Soriano, P. *Cell* **87,** 227–239 (1996).
18. Beckerle, M. C. *Cell* **95,** 741–748 (1998).
19. Laurent, V. *et al. J. Cell Biol.* **144,** 1245–1258 (1999).
20. Lanier, L. M. *et al. Neuron* **22,** 313–325 (1999).
21. Castellano, F. *et al. Curr. Biol.* **9,** 351–360 (1999).

TAPping into transport

Caterina Strambio de Castillia and Michael P. Rout

Studies of viral RNA nuclear export promise to help elucidate the mechanisms used by the cell to transport its own RNA to the cytoplasm. TAP has been implicated in the export of unspliced retroviral RNA transcripts and may be a new RNA-export factor.

Despite tremendous advances in our understanding of how proteins and RNAs are transported between the nucleus and cytoplasm, the mechanisms of RNA export, with a few exceptions, have remained largely mysterious. Now, three new papers from the groups of Izaurralde, Cullen and Hurt[1–3] show how studying the systems used by simple retroviruses to export their unspliced RNA transcripts might shed light on the general mechanisms of cellular RNA export from the nucleus.

Retroviruses require the efficient export of both unspliced and partially spliced viral RNAs to express their structural proteins and package their genomic RNA into virions. To achieve RNA export, they have to be able to bypass the cellular checkpoints that ensure the nuclear retention of unspliced transcripts, and then sneak their way into the normal nuclear export pathway.

In the case of human immunodeficiency virus type 1 (HIV-1), a 'complex' retrovirus, the viral RNA is first bound by Rev, a viral protein. Rev has a nuclear export sequence (NES) that is recognized by Crm1, a nuclear transport factor that belongs to the karyopherin (also known as the importin, exportin or transportin) family. Crm1 then acts in concert with the small GTPase Ran to effect the translocation of the transport substrate across the nuclear envelope through the nuclear pore complexes (NPCs). We still know surprisingly little about the mechanism by which karyopherins translocate across the NPC, although it seems that Ran is required to supply energy and that the movement is mediated by the binding of karyopherins to regions of particular NPC proteins that contain numerous so-called 'FG' (for phenylalanine–glycine) repeat motifs.

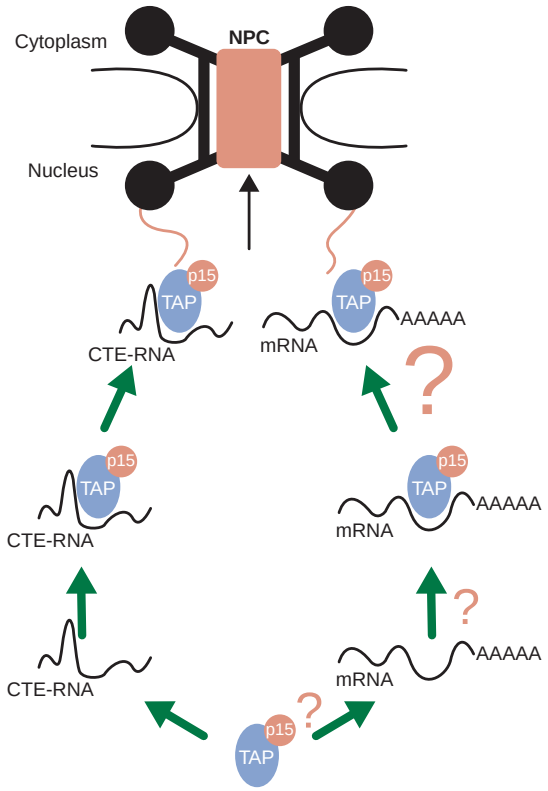

Figure 1 **Is TAP a global mRNA export factor? TAP recognizes the constitutive transport element (CTE) sequence present in retroviral RNAs and mediates their export from the nucleus in a largely unknown manner. TAP appears to be independent of the action of known nuclear transport factors and to interact directly with nuclear pore complexes (NPCs). TAP binds to p15, which could cooperate with TAP to promote CTE binding and NPC association. TAP also binds to cellular mRNA. Moreover, TAP is the closest human relative of the yeast protein Mex67, which is implicated in mRNA export. In the model proposed, TAP interacts with cellular mRNAs and effects their translocation through the NPC by a mechanism similar to the one used by TAP to export viral RNAs.**

'Simple' retroviruses, on the other hand, do not encode specific proteins for viral RNA export and thus need to hijack other cellular factors to achieve the same goal. One well characterized example is that of the simian D-type retroviruses, whose unspliced transcripts possess a sequence termed the constitutive transport element (CTE) that promotes their export from the nucleus. Although the function of the CTE has been recognized for a while, it was only recently that the host-cell protein TAP was identified as a CTE-binding protein that mediates the nuclear export of CTE-containing RNAs (Fig. 1)[4], raising the possibility that TAP is itself a nuclear export factor for RNAs.

However, for a protein to be considered an RNA-export factor it should fulfil some important prerequisites. First, it has to be able to induce directly the movement of its substrate RNA out of the nucleus. Second, in order to do so it has to be able to bind its cognate RNA either directly, or indirectly through an adaptor. Last but not least, it has to move out of the nucleus with the substrate by interacting directly with the NPC, and then move back inside the nucleus ready for the next round of transport; in other words it has to be able to shuttle back and forth between nucleus and cytoplasm. Until now, it was not known whether TAP possessed these attributes.

Together, the three new papers show that TAP does indeed possess all of these characteristics and also assign functions to individual domains of the protein[1–3]. Kang and Cullen[2] show directly that TAP is sufficient to promote CTE-dependent export of RNA by demonstrating that cultured quail cells, normally impervious to the primate viral CTE function, are rendered permissive to this system by the addition of human TAP[2]. Also, this group as well as Braun *et al.*[1] mapped the CTE-binding domain of TAP to a central, leucine-rich segment of the protein by using a combination of *in vitro* and *in vivo* binding studies. As expected, this domain of TAP is necessary for CTE-dependent export from the nucleus; moreover, it may not be sufficient for CTE function, indicating that other regions of the protein are responsible for important aspects of the action of TAP.

Consistent with this view, two regions of TAP involved in its shuttling in and out of the nucleus have now been identified and shown to be important for CTE function[2,3]. A sequence at the amino terminus increases the efficiency of TAP's nuclear uptake whereas a second region, at the carboxy terminus, appears to contain the main nuclear import and export determinants. The mechanism of action of these localization domains is still unclear, but it is possible that the C-terminal region may promote the association of TAP with the NPCs and, in this way, direct its movement in and out of the nucleus, while the N-terminal domain could act as a secondary system to increase the efficiency of TAP recycling.

A large body of circumstantial evidence points to the possibility that TAP might represent a nuclear transport factor that does not belong to the karyopherin family but still carries out an analogous function. Various experiments indicate that TAP may use an export route different to that used by Crm1 and other karyopherins. Thus, inhibitors of Crm1 do not inhibit CTE-mediated export[5,6]. Also, microinjection experiments in *Xenopus* oocytes show that large levels of CTE do not inhibit Rev-mediated export, and that large amounts of the Rev NES (which inhibit the export of the Rev RNA substrate) have no effect on CTE function[7,8]. Furthermore, CTE-dependent export may use the energy source of Ran in a different way to the karyopherins involved in known export processes; microinjection into *Xenopus* oocytes of a particular Ran mutant does not block the export of various karyopherin substrates, including Rev, transfer RNAs and karyopherin/importin-α. However, this mutant efficiently blocks CTE export[8,9].

Together, these results strongly indicate that TAP might be a *bona fide* RNA-export factor, although the possibility cannot be excluded that an atypical and as-yet-unidentified karyopherin might do the job by recognizing TAP as its export substrate (however unlikely this now seems).

Another line of supporting evidence is the observation that TAP interacts with a new protein called p15, and that p15 is required for at least some aspects of TAP function[3]. p15 shares some sequence similarities with NTF-2, a Ran-binding protein that is essential for nuclear import of proteins. Although this might suggest that p15 recruits Ran into action to promote TAP-dependent export, a direct interaction between p15 and Ran has not yet been detected[3].

But the strongest support for the idea that TAP is an RNA-export factor comes from the finding that TAP localizes to the NPCs *in vivo*, and binds directly to the FG-repeat-containing region of NPC proteins through its C-terminal domain[3]. The target of this interaction appears to be at least the FG-repeat regions of CAN/Nup214 and also of a new human potential NPC protein, hCG1. Thus TAP does not need a karyopherin to dock specifically to the NPC.

Clearly, nuclear export of viral unspliced RNAs can be mediated by TAP; however, a key question is whether TAP is also involved in cellular RNA export. Interestingly, work initiated in yeast by one of the three groups has converged on TAP[3], providing crucial circumstantial evidence that points to the involvement of this protein in export of cellular messenger RNA.

Yeast possesses a protein, called Mex67, with significant sequence similarities to TAP; Mex67 has been implicated in export of poly(A)$^+$ RNA transcripts but seemingly not of tRNA and ribosomal RNA[10]. A null mutation of the gene encoding Mex67 renders the cell unable to export mRNA from the nucleus and eventually kills the

cell, indicating that Mex67 has something to do with this process[10]. Also, Mex67 is found in a complex with Mtr2, a second protein thought to be involved in mRNA export[10,11]. Further evidence of the involvement of Mex67 in mRNA export is provided by the observation that the Mex67–Mtr2 complex binds directly to mRNA both *in vivo* and *in vitro*[10,11]. Finally, both Mex67 and Mtr2 are localized to the NPC and have been found to interact with NPC proteins[10,11].

Interestingly, mapping studies done with Mex67 show that the domain organization of this protein is similar to that of TAP. But Katahira *et al.*[3] furnish the strongest indication that TAP and Mex67 carry out similar functions by showing that human TAP, in combination with p15, can rescue a *mex67 mtr2* double null mutation, although it is interesting that Mtr2 and p15 do not share any obvious sequence similarities[3]. All of these data, together with the observations that TAP itself binds mRNA and that CTE microinjection in *Xenopus* oocytes interferes with mRNA export[1,3,4,7], indicate that TAP might be involved in mRNA export (Fig. 1).

Of course, there is still much to be resolved. For example, there are some discrepancies between Braun *et al.*'s and Kang and Cullen's reports[1,2] about the exact definition of the minimal functional unit of the CTE and about the ability of the CTE-binding domain of TAP to promote CTE-dependent RNA export on its own[1,2]. It is possible that these differences are due to the use of different experimental systems and therefore the presence of different accessory factors. Another open question asks what happens to the viral RNA after it is spliced and whether spliced RNA also requires TAP at some point in its export pathway.

Most important, the true cellular role or roles of TAP are far from having been established. For instance, in the case of CTE-dependent export, TAP clearly behaves as a specific RNA-binding protein. On the other hand, in the case of global mRNA export TAP's binding to RNA would have to be less specific. In this context, it will be interesting to determine whether Mtr2 and p15 can function to modify TAP's binding affinity for different kinds of RNA, or whether the two TAP homologues present in the human genome could be involved in transporting different classes of transcript.

Although TAP has still not been caught red-handed taking cellular RNA out of the nucleus, it has at least been found with a viral accomplice, lingering at the scene of the crime, and holding the stolen goods. Further interrogation of TAP will surely provide the incriminating evidence, leading to a better understanding of the mechanisms used by the cell to transport RNA from its site of synthesis to its site of action.□

Caterina Strambio de Castillia and Michael P. Rout are at the Laboratory of Cellular and Structural Biology, Box 213, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.
e-mail: rout@rockvax.rockefeller.edu

1. Braun, I. C., Rohrbach, E., Schmitt, C. & Izaurralde, E. *EMBO J.* **18,** 1953–1965 (1999).
2. Kang, Y. & Cullen, B. *Genes Dev.* (in the press).
3. Katahira, J., Strasser, K., Podtelejnikov, A., Mann, M., Jung, J. U. & Hurt, E. *EMBO J.* (in the press).
4. Gruter, P. *et al. Mol Cell* **1,** 649–659 (1998).
5. Bogerd, H. P., Echarri, A., Ross, T. M. & Cullen, B. R. *J. Virol.* **72,** 8627–8635 (1998).
6. Otero, G. C., Harris, M. E., Donello, J. E. & Hope, T. J. *J. Virol.* **72,** 7593–7597 (1998).
7. Pasquinelli, A. E. *et al. EMBO J.* **16,** 7500–7510 (1997).
8. Saavedra, C., Felber, B. & Izaurralde, E. *Curr. Biol.* **7,** 619–628 (1997).
9. Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, I. W. & Gorlich, D. *EMBO J.* **16,** 6535–6547 (1997).
10. Segref, A. *et al. EMBO J.* **16,** 3256–3271 (1997).
11. Santos-Rosa, H. *et al. Mol. Cell. Biol.* **18,** 6826–6838 (1998).

How BAD phosphorylation is good for survival

Julian Downward

BAD is a pro-apoptotic member of the Bcl-2 family that is tightly regulated by survival factors. Several major signalling pathways influence cell death through their direct effects on the phosphorylation state of BAD.

The regulation of programmed cell death — apoptosis — is an exceptionally complicated process that involves a myriad of proteins. The gene encoding one of these proteins, Bcl-2, was originally identified as a tumour-promoting gene, or oncogene, that was overexpressed in human follicular B-cell lymphomas as a result of its translocation from chromosome 18 to chromosome 14 (ref. 1). It later became clear that the Bcl-2 protein was oncogenic not because it promoted uncontrolled cell growth but because it blocked programmed cell death. A large family of related proteins, numbering 16 so far in mammals, has now been identified; this family is divided into three subfamilies, consisting of proteins that are most similar to Bcl-2 and are anti-apoptotic (Bcl2-like proteins), those that have structures related to that of Bcl-2 but are pro-apoptotic (Bax-like proteins), and more distantly related proteins that share only one region of homology with Bcl-2, the BH3 domain, and are pro-apoptotic (BH3-only proteins). These proteins have emerged as key players in the regulation of apoptosis and have been proposed to integrate signals from survival-inducing and death-promoting pathways. In particular, much interest has focused recently on BAD, a BH3-only pro-apoptotic protein, which is inactivated by survival-promoting factors, such as interleukin-3 (IL-3), acting in part through protein kinase B (PKB; also known as Akt) (Fig. 1)[2]. Two papers have now provided new insight into the control of BAD, with the identification of a second protein kinase that can inactivate BAD[3] and a phosphatase that can reactivate it[4].

BAD was first identified because of its ability to bind to Bcl-2 and Bcl-X_L[5] — members of the Bcl2-like subfamily of Bcl-2 proteins. Bcl-2 and Bcl-X_L exert their anti-apoptotic effect, at least in part, by binding to Bax and related pro-apoptotic proteins and preventing them from inducing damage to mitochondria that would result in release of cytochrome *c* and activation of the caspase-9/Apaf-1 'apoptosome'. Interaction of Bcl-2 or Bcl-X_L with BH3-only proteins, such as BAD, sequesters Bcl-2 and Bcl-X_L away from the Bax-like proteins and thus promotes apoptosis, even though BAD doesn't appear to have any direct ability to induce the release of cytochrome *c* from mitochondria. Consistent with this idea, BAD lacks any transmembrane sequence, unlike most members of the family[13].

Further work[6] showed that the treatment of lymphoid progenitor cells with IL-3 resulted in the phosphorylation of BAD at two sites, serine residues 112 and 136 (this numbering is based on the sequence of murine BAD). Phosphorylation at either of these residues created consensus sites for interaction with the 14-3-3 protein; BAD then bound to 14-3-3 instead of Bcl-2 or Bcl-X_L, resulting in the liberation of the anti-apoptotic proteins and the consequent promotion of cell survival. BAD protein in

