ATP transport and ABC proteins

Guido Guidotti

ATP can be exported into the extracellular space, where it has important biological effects. Recent evidence shows that direct ATP export across the plasma membrane is associated with the presence of ABC proteins. Do the ABC proteins pump ATP as well as their other substrates, and if so, why?

Address: Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA.

Chemistry & Biology September 1996, **3**:703–706

 ISSN 1074-5521

ATP binding cassette (ABC) proteins are a large and diverse family of transport proteins that are involved in a number of important biological processes. The multidrug resistance (MDR) protein, also known as P-glycoprotein, belongs to this family; it confers resistance to a wide range of cytotoxic drugs on human cancer cells, by pumping the drugs out of the cell in an ATP-dependent process [1]. Recent evidence suggests that ABC proteins may also be involved in the transport of ATP across membranes. Here I will review the evidence for the presence of ATP in the extracellular fluid and what is known about the ATP transport systems that have previously been identified, before discussing the evidence that ABC proteins are involved in the movement of ATP and the possible biological roles of ATP efflux.

Evidence for extracytoplasmic ATP

The effects of extracellular ATP on many, if not most, organs and cells are well recognized and have been described in considerable detail in recent reviews [2]. The effects are mediated by specific receptors for ATP, the P_2 purinergic receptors. The presence of these P_2 purinergic receptors makes it evident that ATP is present extracellularly in at least some biological circumstances.

How does ATP get into the extracellular fluid? Other than the obvious release of intracellular ATP by disrupted cells, there are two possible sources for extracellular ATP.

ATP transport in organelles

One possible source of ATP is its release from secretory granules and vesicles during their fusion to the plasma membrane. ATP is present in adrenal chromaffin granules at a concentration of 0.13 M along with catecholamines [3], and is copackaged with serotonin in platelet granules and with catecholamines and with acetylcholine in synaptic vesicles. ATP uptake into adrenal chromaffin granule ghosts has recently been measured, and has been reconstituted in proteoliposomes [4]. The unidirectional transport process is driven by the membrane potential, and has a K_M for ATP of 2.9 mM and a V_{max} of 0.12 nmol per mg of ghost protein per minute. It is thus probably not related to the uptake system responsible for net changes in the adenine nucleotide content of mitochondria, which appears to be carried out by an electroneutral ATP-Mg/P_i exchanger with a K_M for ATP of 2–4 mM and a V_{max} of 3.6 nmol min^{-1} mg^{-1} mitochondrial protein [5]. The proteins responsible have not been identified, but are clearly distinct from those involved in the movement of ATP into the endoplasmic reticulum (ER) of yeast [6] and the rough ER of animal cells [7]. The ER transporters seem to work by nucleotide exchange, and may therefore be related to the ATP/ADP exchanger of mitochondria [8]. Mayinger *et al.* [6] reconstituted the yeast microsomal ATP transporter, identifying a 68 kDa transmembrane protein, Sac1p, that is responsible for transport. Sac1p has a K_M for ATP of 11 μM and a V_{max} of 1.2 nmol mg^{-1} min^{-1}. The transporter responsible for ATP accumulation into chromaffin granules thus seems to be different from the previously known systems.

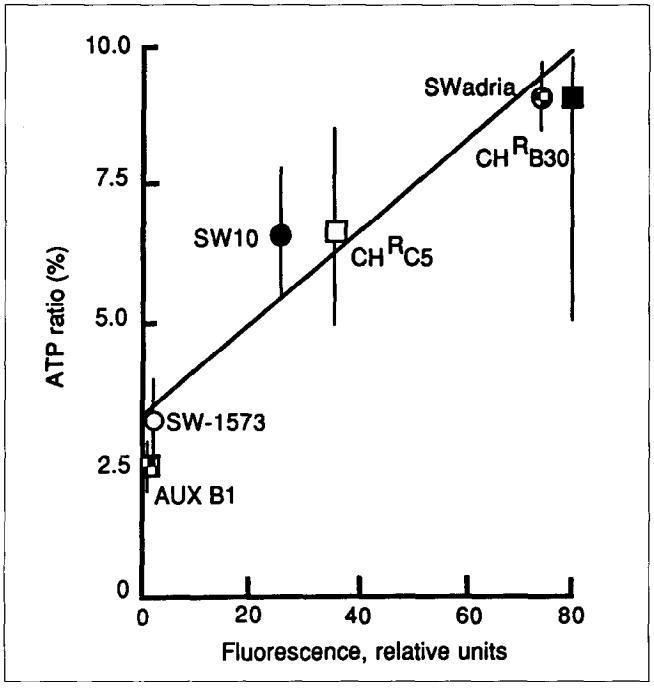
ATP transport across the plasma membrane

A second possible source of extracellular ATP is the movement of cytoplasmic ATP directly across the plasma membrane through transporters or channels. That such movement is possible is supported by the demonstration that cytosolic ATP can be released from cardiac myocytes and red blood cells that are hypoxic, from endothelial cells and smooth muscle cells upon stimulation with catecholamines, and by muscle fibers upon electrical stimulation of the innervated skeletal muscle of the frog [2].

Cells expressing P-glycoprotein release ATP into the medium at a rate proportional to the amount of P-glycoprotein in the plasma membrane (Fig. 1) [9]. CHO cells overexpressing P-glycoprotein extruded ATP at three to four times the rate of the control cells (Fig. 2). In human lung tumor cells expressing P-glycoprotein, the rate of ATP secretion was proportional to the amount of the substrate adriamycin in the medium (E.H. Abraham, personal communication). This result argues strongly for coupling between drug secretion and ATP efflux.

Three important features of these results should be noted. First, ATP is extruded from the parental CHO cells, (AUX B1; Fig. 2), which do not express measurable levels of P-glycoprotein, indicating that other proteins can also catalyze ATP efflux. Second, in cells over-expressing P-glycoprotein, ATP efflux occurs even in the absence of

Figure 1



Correlation between ATP release and plasma membrane level of P-glycoprotein. Cells derived from the parent CHO cell line AUX B1 are shown as squares, those derived from the human lung tumor SW-1573 as circles. 5 x 10^4 cells were attached to 5-mm glass coverslips. The graph plots the relative fluorescence of the cell surface after staining with antibodies to surface epitopes of P-glycoprotein against the amount of ATP determined by luminometry. ATP levels are reported as the percentage of steady-state extracellular ATP relative to the total ATP after release by alamethicin. Reproduced with permission from [9].

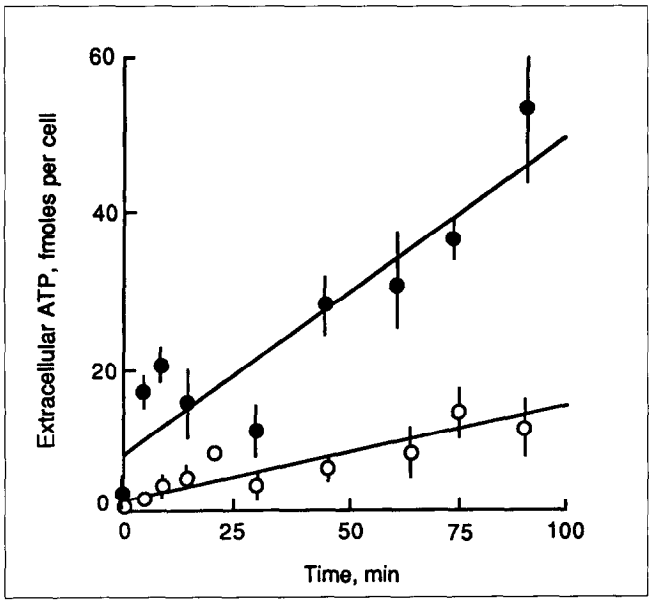
transportable substrate. Thus, either the cell normally contains a transportable substrate, perhaps the natural (unidentified) substrate, for P-glycoprotein, or ATP efflux is a constitutive feature of the activity of P-glycoprotein. P-glycoprotein has constitutive ATPase activity, which is only modestly increased (by two-fold) in the presence of saturating amounts of transportable substrate [10]; the energy thus generated might be used for transporting ATP. Third, the size of the increase in the rate of ATP transport in cells that express P-glycoprotein is interesting. For CH^RC5 cells, this increase is 0.3 fmol per cell per minute, or 4 x 10^6 ATP molecules per cell per second; since these cells have ~10^6 molecules of P-glycoprotein in the plasma membrane, the flux of ATP per P-glycoprotein molecule is ~4 s^{-1}, close to the turnover rate for the hydrolysis of ATP in the absence of transportable substrate.

Although the stoichiometry between the molecules of ATP hydrolyzed by P-glycoprotein and the molecules of drug transported is variable and large [11], the fact that ATP efflux doubles in the presence of adriamycin argues that ATP efflux and drug efflux can be coupled, indicating that P-glycoprotein may well be directly involved in efflux. Whole cells containing the P-glycoprotein in the plasma membrane also were shown to have a 2 nS conductance for ATP with 100 mM ATP on both sides of the membrane [9]; this concentration, however, is not physiological.

Circumstantial support for the view that P-glycoprotein can cause ATP efflux is provided by the finding that most cells naturally expressing P-glycoprotein also have ecto-apyrase activity (T.-F. Wang and G.G., unpublished data). Ecto-apyrases (also called ecto-nucleotidases, ATP-diphosphohydrolases and ecto-ATPases), have been found in the plasma membranes of many cells from different tissues [12], including the apical surface of liver cells which also contain P-glycoprotein [13]. They hydrolyze the terminal phosphate residue of nucleoside triphosphates and diphosphates present in the extracellular fluid, removing their ability to act as ligands for the P_2 purinergic receptors present on the same cell. The coincidence of expression patterns of these two proteins suggests that their activities may be related.

Cells expressing another member of the ABC family of transporters, the cystic fibrosis transmembrane conductance regulator (CFTR), have been reported to export ATP [14] and to have channels for ATP with a conductance of ~5 pS in the plasma membrane [15]. Evidence for the association of ATP transport with CFTR was also provided

Figure 2



ATP release from CHO cells overexpressing P-glycoprotein is significantly more rapid than that of wild-type cells. ATP release was measured from confluent layers of 5 x 10^6 wild-type CHO cells (AUX B1, open circles) or P-glycoprotein expressing cells (CH^RC5, filled circles) using high-pressure liquid chromatography. Bars indicate the standard error of the mean. Inhibitors of adenylate cyclase and ecto-ATPase were added to prevent ATP degradation Reproduced with permission from [9].

by reports from Guggino and colleagues [16] on the activation of outwardly rectifying chloride channels (ORCCs) by extracellular ATP. Not all workers agree, however, that CFTR can conduct ATP [17,18]. Takahashi *et al.* [19] have also reported that cells expressing CFTR do not extrude ATP at a rate different from cells without CFTR, contradicting the previous findings. The explanation for the differences in the results is not obvious, and it is therefore unclear whether the CFTR itself is responsible for the reported ATP export.

Thus, the central question is whether the movement of ATP associated with the presence of the ABC proteins is actually carried out by these proteins or by an associated transport system specific for ATP. There is precedent for the association of ABC proteins with transporters and channels. CFTR seems to form a complex with ORCCs [16] and with a sodium channel [20], and the sulfonylurea receptor, another ABC protein interacts with an ATP-regulated K channel [21]. While it is conceivable that an unidentified ATP channel or transport system can also associate with one or more ABC proteins, it seems more likely that the ABC proteins themselves are responsible for ATP movement.

Role of ATP efflux

If the increased extrusion of ATP from cells expressing P-glycoprotein and other ABC family members is a specific transport property associated with these proteins, not an artefact of some kind, what is its function?

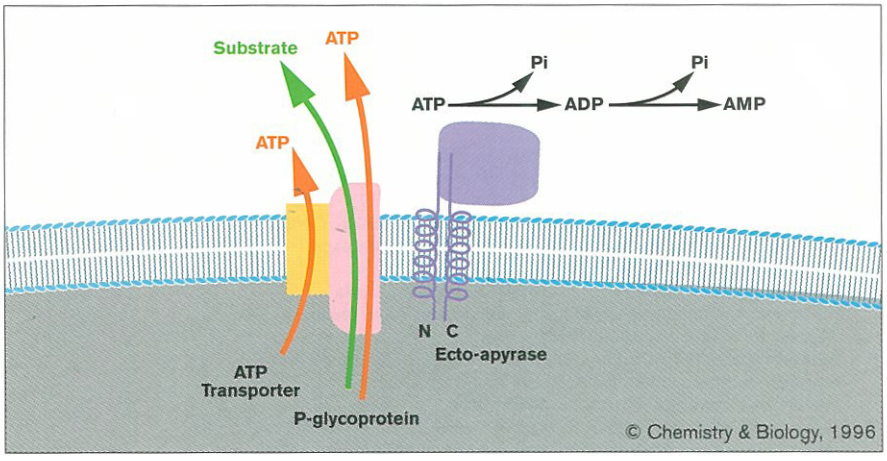
One possibility is that ATP extrusion is part of a signaling system, both autocrine and between cells, that uses ATP as the messenger. In this case it would be expected that the release of ATP would be regulated. This possibility has not yet been explored. Alternatively it may be that ATP secretion is part of the mechanism of transport of some ABC proteins. Thus, the movement of ATP might be coupled to that of the other substrates transported by these proteins, perhaps because ATP associates with these substrates.

Yet a third possibility is that the main function of some ABC proteins is to regulate the ATP concentration at the inner surface of the plasma membrane, by exporting excess ATP. According to this view, maintenance of appropriate ATP concentrations at the plasma membrane is critical for the cell; ATP is required to fuel the Na^+,K^+-ATPase and other plasma membrane enzymes that use ATP, for instance adenylyl cyclase and various kinases, and to regulate the activity of ATP-gated K channels, for instance the β-cell inward rectifier (BIR), which is regulated by the sulfonylurea receptor [21,22]. Regulation of ATP levels would be the result of a balance between the level of glycolysis, which produces ATP that is preferentially used at the plasma membrane [23], and the activity of an ABC protein that extrudes ATP to the extracellular fluid. In support of this view is the observation that animal cells are not unique in their ability to extrude ATP to the extracellular fluid. Yeast cells constitutively secrete ATP into the extracellular fluid and the rate of efflux is increased 10-fold by the presence of protonophores in the medium (R. Boyum and G.G., unpublished results). Interestingly, the rates of ATP efflux per surface area are similar for the P-glycoprotein-expressing CHO line, CH^RC5, and for yeast cells in the presence of nigericin. P-glycoprotein and possibly other ABC proteins may have originally evolved as ATP carriers; subsequently, they may have been adapted to cotransport other molecules that can interact with ATP.

Although it might seem unlikely that the concentration of an important molecule like ATP would be regulated by excretion, this type of regulation is not unprecedented. cAMP is vigorously secreted from cells when its production is increased [24]. Furthermore, over 90 % of the cAMP produced by *Escherichia coli* [25] and yeast [26] is present in

Figure 3

A proposal for the organization of a putative multiprotein system for regulating extracellular ATP levels, composed of an ecto-apyrase, P-glycoprotein and a putative ATP translocator. It is not known whether ATP moves through the P-glycoprotein (and other ABC proteins) itself or through an associated ATP translocator. The extracellular ATP is eventually degraded by the ecto-apyrase, limiting its signaling.



the extracellular fluid. Excretion may be a simple way to regulate the intracellular concentration of cAMP at the plasma membrane, which cannot be degraded rapidly enough by phosphodiesterases. Export of cAMP is a well documented event; however, the mechanism of the process is not known.

Conclusions

Extracellular ATP has many functions: it is a ligand for P_2 purinergic receptors and possibly for protein kinases, and can be converted into adenosine, a ligand for P_1 purinergic receptors. The question of how ATP is extruded into the extracellular space is a fascinating and potentially important one. The possibility that the ABC proteins may directly mediate ATP transport across the plasma membrane (Fig. 3) is intriguing; it may offer insight into the mechanism of action of the ABC proteins and, perhaps, into their evolutionary history.

References

1. Gottesman, M.M. and Pastan, I. (1993). Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62**, 385–427.
2. Dubyak, G.R. & El-Moatassim, C. (1993). Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. *Am. J. Physiol.* **265**, C577–C606.
3. Winkler, H. & Carmichael, S.W. (1982). The chromaffin granule. In *The Secretory Granule.* (Poisner, A.M. & Trifaro, J.M., eds), pp. 3–79. Elsevier, Amsterdam
4. Bankston, L.A. & Guidotti, G. (1996). Characterization of ATP transport into chromaffin granule ghosts. Synergy of ATP and serotonin accumulation in chromaffin granule ghosts. *J. Biol. Chem.* **271**, 17132–17138.
5. Joyal, J.L. & Aprille, J.R. (1992). The ATP-Mg/P_i carrier of rat liver mitochondria catalyzes a divalent electroneutral exchange. *J. Biol. Chem.* **267**, 19198–19203.
6. Mayinger, P., Bankaitis, V.A. & Meyer, D.I. (1995). Sac1p mediates the adenosine triphosphate transport into yeast endoplasmic reticulum that is required for protein translocation. *J. Cell Biol.* **131**, 1377–1386.
7. Guillen, E. & Hirschberg, C.B. (1995). Transport of ATP into ER proteoliposomes. *Biochemistry* **4**, 5472–5476.
8. Klingenberg, M. (1985). The ADP/ATP carrier in mitochondrial membranes. In *The Enzymes of Biological Membranes Vol. 4.* (Martinosi, A.N., ed.), pp. 511–553, Plenum Press, New York.
9. Abraham, E.H., *et al.*, & Cantiello, H.F. (1993). The multidrug resistance (*mdr1*) gene product functions as an ATP channel. *Proc. Natl. Acad. Sci. USA* **90**, 312–316.
10. Urbatsch, I.L., Al-Shawi, M.K. & Senior, A.E. (1994). Characterization of the ATPase activity of purified Chinese hamster P-glycoprotein. *Biochemistry* **33**, 7069–7076.
11. Shapiro, A.B. & Ling, V. (1995). Reconstitution of drug transport by purified P-glycoprotein. *J. Biol. Chem.* **270**, 16167–16175.
12. Plesner, L. (1995). Ecto-ATPases: identity and functions. *Intern. Rev. Cytol.* **158**, 141–214.
13. Kamimoto, Y., Gatmaitan, Z., Hsu, J. & Arias, I.M. (1989). The function of Gp170, the multidrug resistance gene product, in rat liver canalicular membrane vesicles. *J. Biol. Chem.* **264**, 11693–11698.
14. Prat, A.G., Reisin, I.L., Ausiello, D.A. & Cantiello, H.F. (1996). Cellular ATP release by the cystic fibrosis transmembrane conductance regulator. *Am. J. Physiol.* **270**, C538–C545.
15. Reisin, I.L., et al., & Cantiello, H.F. (1994). The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J. Biol. Chem.* **269**, 20584–20591.
16. Schwiebert, E.M., *et al.*, & Guggino, W.B. (1995). CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* **81**, 1063–1074.
17. Reddy, M.M., et al., & Kopito, R.R. (1996). Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. *Science* **271**, 1876–1879.
18. Li, C., Ramjeesingh, M. & Bear, C.E. (1996). Purified cystic fibrosis transmembrane conductance regulator (CFTR) does not function as an ATP channel. *J. Biol. Chem.* **271**, 11623–11626.
19. Takahashi, T., Matsushita, K., Welsh, M.J. & Stokes, J.B. (1994). Effect of cAMP on intracellular and extracellular ATP content of Cl secreting epithelia and 3T3 fibroblasts. *J. Biol. Chem.* **269**, 17853–17857.
20. Stutts, M.J., *et al.*, & Boucher, R.C. (1995). CFTR as a cAMP-dependent regulator of sodium channels. *Science* **269**, 847–850.
21. Inagaki, N., *et al.*, & Bryan, J. (1995). Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270**, 1166–1170.
22. Al-Awqati, Q. (1995). Regulation of ion channels by ABC transporters that secrete ATP. *Science* **269**, 805–806.
23. Campbell, J.D. & Paul, R.J. (1992). The nature of fuel provision for the Na,K-ATPase in porcine vascular smooth muscle. *J. Physiol.* **447**, 67–82.
24. Brunton, L.L. & Heasley, L.E. (1988). cAMP export and its regulation by prostaglandin A1. *Methods Enzymol.* **159**, 83–92.
25. Matin, A. & Matin, M.K. (1982). Cellular levels, excretion, and synthesis rates of cyclic AMP in *E. coli* grown in continuous culture. *J. Bacteriol.* **149**, 801–807.
26. Smith, M.E., Dickinson, J.R. & Wheals, A.E. (1990). Intracellular and extracellular levels of cyclic AMP during the cell cycle of *Saccharomyces cerevisiae. Yeast* **6**, 53–60.