Novel drug development strategy targets protein 'export'

A key protein sequence that enables fibroblast growth factor 2 (FGF-2) to exit cancer cells and stimulate angiogenesis and tumour growth has been identified by scientists at Ciblex (San Diego, CA, USA). Andrew Baird (Vice-President R&D, Ciblex) confirms that a programme of preclinical development is underway to identify molecules that can selectively block cellular release of proteins, such as FGF-2, that leave the cell via a process termed 'protein export' or 'protein translocation'[1].

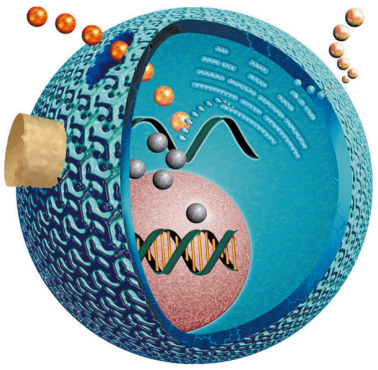


Figure 1. *Protein export. The secretion pathway, in which proteins are inserted into the endoplasmic reticulum, packaged into the Golgi apparatus and released from the cell surface membrane in vesicles, is shown on the right. The cell membrane transport apparatus that exports leaderless proteins is illustrated on the left. In this cell, the protein export process has been blocked, and the leaderless protein cannot leave the cell.*

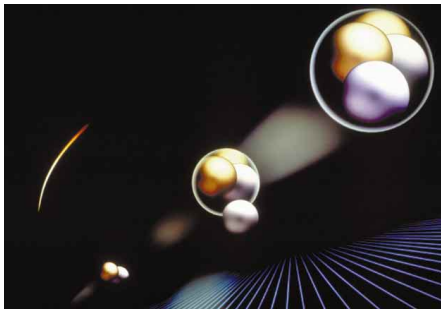
Protein export – a therapeutic target

Protein export is an alternate, endoplasmic reticulum/golgi-independent exocytic pathway discovered by several researchers in the early 1980s (Ref. 1; Fig. 1). Baird and long-time collaborator, Robert Florkiewicz (both co-founders of Ciblex), remember that the theory that an alternative to secretion might exist threatened to overturn pre-existing dogma. 'For that reason, the idea was accepted slowly at first, but it is now beginning to be recognized as a distinct cell transport process that might be manipulated to therapeutic advantage,' says Baird.

It was originally thought that all proteins released from the cell in a controlled process left via the endoplasmic reticulum–golgi pathway, because all proteins known to be secreted had hydrophobic leader sequences that facilitated their movement through plasma membranes. 'We then found that FGF, a protein that was known to be released from cells, had no leader sequence,' adds Baird.

Initially, the most likely theory was that the protein was released during apoptosis, and that no particular mechanism was responsible. However, over the next few years, several more proteins without leader sequences were discovered in other laboratories, including mammary-derived growth inhibitor, thioredoxin, interleukin-1 (IL-1) and macrophage migration inhibitory factor (MIF)[2]. Hence, a second less-likely theory, that another release mechanism was operating for some proteins, started to gain support.

Cell-based assays identify selective inhibitors



Experimental evidence followed from cell-based assays in which researchers looked for small molecules that could selectively block either the secretion pathway, leaving the alternative pathway unchanged, or vice versa. Both mechanisms turned out to be possible. In 1998, Baird and colleagues reported that they had identified a family of related compounds that could inhibit FGF-2 export in a time- and concentration-dependent fashion. Inhibition was specific: the inhibitors had no effect on conventional protein secretion as measured by their inability to block release of the secreted protein human chorionic gonadotrophin-α (Ref. 3).

To date, 24 proteins without leader sequences have been discovered, and all are thought to be released from cells via the export process. 'What is not known yet is whether there are many more such proteins, or whether the export process acts for only these rare cases,' observes Baird. The molecular mechanisms involved in the export process remain to be characterized. However, there is some evidence that the α-subunit of the Na^+/K^+-ATPase is involved in FGF-2 export and it has been suggested that this subunit might itself be an integral component of this alternate exocytic pathway[3].

Export is specific

1359-6446/00/$ – see front matter PII: S1359-6446(00)01542-7

A surprising recent finding demonstrated that the export process shows a high degree of specificity. 'When we experimented with glioblastoma cells in culture – cells that normally export FGF-2 – and we put FGF-1 into them, we found that the cells could not release the FGF-1,' reports Baird. Further investigations led to the discovery of the part of the sequence of FGF-2 that might facilitate its export from the cell. Baird and colleagues systematically combined different portions of the protein sequences from FGF-2 and FGF-1 and, by tracking the transport of each FGF-2–FGF-1 chimera through U87-MG glioblastoma cells, identified a specific export signalling sequence within FGF-2.

Baird speculates that cells might have specific export channels for different proteins. This suggests that different export channels might be selectively blocked, opening the possibility of different therapeutic strategies. 'We are now concentrating on a family of molecules – exhibins – that block the IL-1 export process and are investigating them for specific blocking activity using cell-based assays,' says Baird. Chemical libraries are being used to screen analogues within the exhibin family to identify potential drug candidates that work in culture. 'This is a drug discovery/validation programme at the moment,' stresses Baird. The exhibins show promise *in vitro* and are now being tested *in vivo*. One compound (CBX10913) is orally active in mice. 'At the same time, we are also working with other groups to define the molecular pathway involved in export,' adds Baird.

Future therapeutic potential

Drugs that could block protein export would have several important therapeutic uses, but work is progressing particularly well in the areas of inflammatory diseases and cancer. Exported proteins such as IL-1 and IL-18, FGF-1, FGF-2 and MIF play a central role in inflammatory diseases where they are involved in angiogenic, allergic, autoimmune and inflammatory responses that promote cell growth and reactivity. The drug candidates, CBX10913, CBX18469 and CBX21404, which show activity in preclinical models of inflammatory disease, could have potential in rheumatoid arthritis. Exhibins that inhibit the release of the exported proteins from cells are also being investigated for their potential to control autocrine (glioblastoma, melanoma) and paracrine (prostate and breast cancers) cell growth. Compounds CBX11030 and CBX10913 are currently under further evaluation in preclinical cancer models.

'Also of great interest, is the possibility of using exhibins against infectious disease,' says Baird. Gram-negative bacteria have their own protein 'export' pathway – type III secretion – that is responsible for the release of toxins. By interrupting the release of these virulence factors, it might be possible to also develop specific exhibins as novel antimicrobial agents.

REFERENCES

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A new target for HIV-1 entry inhibition?

Despite the major impact of protease and reverse transcriptase inhibitors on HIV-1 treatment in recent years, these drugs cannot eradicate HIV-1 from infected humans. New research has described structural information on the HIV binding site that might provide a new target for the development of HIV-1 entry inhibitors. A collaboration between the Albert Einstein College of Medicine (Bronx, NY, USA), Rockefeller University (New York, NY, USA) and Progenics Pharmaceuticals (Tarrytown, NY, USA) demonstrated that HIV binds to a peptide corresponding to the

amino terminal domain of CCR5 and that this binding is dependent on the presence of sulfotyrosines at certain locations within the peptide[1].

Binding of the HIV-1 envelope glycoprotein gp120 with CD4 exposes or creates a coreceptor binding site that usually interacts with either CCR5 or CXCR4, mediating the entry of R5 and X4 HIV isolates, respectively[2,3]. Furthermore, amino acids 2–18 in CCR5 protein contain all the residues important for viral entry[4]. However, after synthesizing the peptides corresponding to these amino acids, the team, led by Tanya Dragic (Albert Einstein College of Medicine), found that they did not bind to gp120.

Sulfation of the tyrosine residues

Recent work has shown that the tyrosine residues in the CCR5 nucleotide

 1359-6446/00/$ – see front matter PII: S1359-6446(00)01549-X