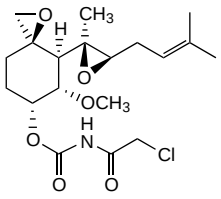
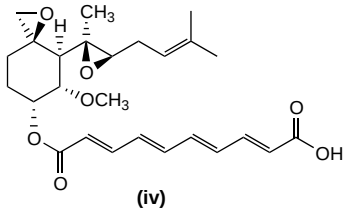


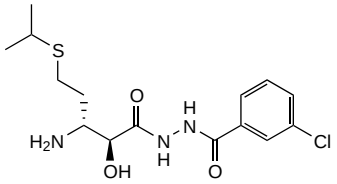
(iii)



(iv)

responsible for removing the NH_2-terminal initiator methionine residue from nascent proteins. MetAPs also play a role in protein cotranslational and/or post-translational modifications, and in maintaining protein stability. Notably, MetAP2 (but not MetAP1) is upregulated during cell proliferation, and inhibition of MetAP activity is thought to affect protein activity, subcellular localisation and degradation, leading to signal transduction and cell cycle interference.

TNP-470 irreversibly inhibits MetAP2 through formation of a covalent adduct (epoxide alkylation by an active site His231 residue), and might alkylate additional proteins, leading to the toxic side effects shown by this drug. Wang and co-workers have now reported a rationally designed (via analysis of enzyme-inhibitor complex crystal structure and parallel synthesis) reversible MetAP2 enzyme inhibitor (A-357300, **v**) with potent and selective MetAP2 inhibition ($IC_{50} = 0.12\ \mu M$) over MetAP1 inhibition ($IC_{50} = 57\ \mu M$) [6]. A-357300 selectively induces cytostasis by G_1 cell cycle arrest in endothelial cells and in a subset of tumour cells, but not in most primary cells of non-endothelial type. In addition, inhibition of angiogenesis (both *in vitro* and *in vivo*) and potent antitumour



(v)

efficacy in carcinoma, sarcoma and neuroblastoma murine models was observed, establishing that reversible MetAP2 inhibitors could have value as novel cancer therapeutic agents.

5 Griffith, E.C. *et al.* (1997) Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin. *Chem. Biol.* 4, 461–467

6 Wang, J. *et al.* (2003) Tumor suppression by a rationally designed reversible inhibitor of methionine aminopeptidase-2. *Cancer Res.* 63, 7861–7869

Andrew D. Westwell

e-mail: andrew.westwell@nottingham.ac.uk

Biology

Cancer biology

Chemical modulation of ER: a novel strategy for the treatment of breast cancer

Since the early 1970s, Tamoxifen has been widely prescribed for the treatment of breast cancer. This drug acts by competing with the natural ligand for binding to the estrogen receptor (ER), thereby modifying ER activity. However, Tamoxifen is only effective in tumours that retain ER expression and/or function and acquired drug resistance is a common occurrence. Thus, novel compounds are requried to circumvent this problem.

Wang *et al.* [1] have taken the innovative approach of targeting the ER DNA-binding domain. This region of the protein comprises two non-equivalent zinc finger motifs that act in concert to mediate ER dimerization and DNA binding. The authors proposed that disruption of the zinc fingers might perturb ER function. To test this hypothesis they analyzed the anti-ER activity of several electrophilic agents. The disulphide benzamine, DIBA, and the benzisothiazolone, BITA, inhibited estradiol stimulated proliferation of ER-positive, but not ER-negative, cell lines; however, other electrophilic agents were ineffective. Similar results were also observed *in vivo* when nude mice harbouring breast carcinoma xenografts were treated with the compounds. At the molecular level, gene analysis demonstrated reduced E2F and anti-apoptosis gene expression, with a concomitant increase in cell cycle inhibitory genes.

The results of this study are encouraging because they demonstrate that it is possible to selectively modulate ER function by disrupting the DNA-binding domain. This might prove a tenable alternative to targeting the ER ligand-binding domain, a strategy that has been associated with acquired drug resistance. Thus, although further work is required to evaluate selectivity, potency and toxicity, the use of electrophilic agents represents a novel approach for the treatment of ER-positive breast cancers.

1 Wang, L.H. *et al.* (2004) Suppression of breast cancer by chemical modulation of vulnerable zinc fingers in estrogen receptor. *Nat. Med.* 10, 40–47

Victoria Heath

vjh2r@udcf.gla.ac.uk

Function of *BLM* – the gene mutated in Bloom's syndrome

Mutations in a human gene, *BLM*, cause a rare genetic disorder known as Bloom's syndrome. This condition is typically characterized by increased genomic instability and cancer predisposition.

The *BLM* gene encodes a DNA helicase belonging to the evolutionarily conserved RecQ family of DNA helicases. BLM interacts with and stimulates the activity of hTOPIIIα, a type IA topoisomerase also implicated in homologous recombination that can catalyze the unlinking of DNA molecules. Wu and Hickson [2] investigated the ability of recombinant BLM and hTOPIIIα proteins to resolve a synthetic oligonucleotide resembling two DNA molecules intertwined into a

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recombination intermediate known as a double Holliday junction.

In this study, the synthetic double Holliday junction substrate was designed so that one DNA molecule was radiolabelled, enabling any changes to be measured by a change in electrophoretic mobility. Additionally, any crossing-over could be detected by following the fate of unique restriction sites on the DNA sequences flanking the double Holliday junction.

Intriguingly, the authors observed that BLM and hTOPIIIα together resolved the double Holliday junction, whereas neither BLM nor hTOPIIIα could achieve this alone. Furthermore, this always occurred without any crossing over of genetic material. This might help explain the high sister chromatid exchange frequency seen in Bloom's syndrome cells, as recombination intermediates might be processed aberrantly in the absence of BLM resulting in abnormally high levels of crossing over. These findings also suggest that suppression of crossing over during homologous recombination is important for maintenance of genomic stability and cancer prevention in normal cells.

2 Wu, L. and Hickson, I.D. (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426, 870-874

Hocine H. Mankouri
h.mankouri@cancer.org.uk

Live action telomeres

Telomeres, at the ends of eukaryotic chromosomes, consist of multiple tandem copies of the hexanucleotide, TTAGGG, together with a complex of associated proteins. These structures perform essential roles for the cell, including maintaining chromosome stability and regulating proliferative life span. In addition, they are responsible for silencing telomere-adjacent genes (by means of their localized heterochromatin structure) and are thought to influence specific chromosome positioning in the nucleus, through their firm attachments to the nuclear matrix. Despite this attachment, new research now reveals that some telomeres, at least, can be surprisingly mobile.

To study telomere movement, Molenaar *et al.* [3] have now established a method of visualizing telomeres in live cells. Using a fluorescent peptide-nucleic acid probe (PNA) the team targeted telomeric repeat sequences in human osteosarcoma cells and studied their motion over time. They observed three different modes of telomere movement. First, the majority of telomeres showed slow (average: 1.8 x 10^{-4} μmetre^2 per s) and constrained diffusion (radius of constraint 0.5 mm), as might be expected for matrix attached DNA. The second group, however, making up about 10% of telomeres, moved considerably faster (average: 5.8 x 10^{-4} μmetre^2 per s) and had a larger radius of confinement (1.2 mm). The third and smallest population moved faster still, with an average speed of 1.9 x 10^{-3} μmetre^2 per s and in the time period analysed had moved so far that a radius of constraint could not be calculated.

The authors suggest that the finding that some telomeres are relatively free from constraint might indicate their dissociation from the nuclear matrix. The authors further suggest that this dissociation might be coupled with transcriptional derepression of telomere-silenced genes. Both of these speculations, however, remain to be tested.

3 Molenaar, C. *et al.* (2003) Visualizing telomere dynamics in living mammalian cells using PNA probes. *EMBO J.* 22, 6631–6641

Ruth R. Williams
ruth.williams@csc.mrc.ac.uk

Virology

The type V secretion pathway: a premium source of virulence factors?

Since its discovery in the late 1980s, the family of secreted proteins termed the autotransporters has been expanding continuously to become the largest group of secreted proteins in Gram-negative bacteria. The type V secretion pathway, which includes the autotransporters, can be defined by secreted proteins that are: (i) translocated across the outer membrane via a transmembrane pore formed by a β-barrel; and (ii) contain all the information required for translocation through the cell envelope.

The autotransporters are restricted to the phylums Proteobacteria and *Chlamydiae*. By characterizing the polymorphic membrane protein PmpD from *Chlamydophila pneumoniae*, Wehrl *et al.* [5] demonstrate that it is a truly autotransporter protein. The neosynthetised PmpD is exported from the cytoplasm to the periplasmic space by the Sec apparatus with the concomittant cleavage of the N-terminal signal sequence. The C-terminal part of the protein then forms a β-barrel in the outer membrane allowing secretion of the N-terminal passenger domain outside the cell. It was hypothesized that the protein would interact with some components of the outer membrane.

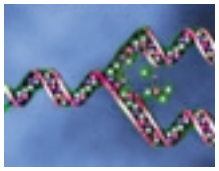
All autotransporter proteins characterized thus far act as virulence factors. PmpD shares homology with some adhesins; this is supported by the presence of a highly repetitive tetra-amino acid motif involved in adhesion to membranes of different host cell types. Such a function is suggested experimentally by neutralizing chlamydial infectivity with anti-N-pmpD antibodies. However, this finding does not discriminate between an inhibition of binding and uptake/invasion, and a negative effect of bound antibodies on the course of chlamydial development after entry into the eukaryotic cell.

Although further work is needed to clarify this, the direct interaction of PmpD with the host cells and the subsequent mediation of immunostimulatory events demonstrate its role as a key virulence factor. From a therapeutic point of view, it demonstrates further that PmpD is an important target for anti-chlamydial vaccination.

5 Wehrl, W. *et al.* (2004) From the inside out - processing of the Chlamydial autotransporter PmpD and its role in bacterial adhesion and activation of human host cells. *Mol. Microbiol.* 51, 319–334

Ian R. Henderson
i.r.henderson@bham.ac.uk
Mickael Desvaux
m.desvaux@bham.ac.uk

BRCA2 required to prevent formation of double-strand breaks



The exact role of BRCA2 in tumour suppression is still not clear. Loss-of-function of BRCA2 causes chromosomal instability: when DNA replication is blocked, BRCA2 localizes to the DNA replication machinery but its function in DNA replication is unknown. Lomonosov *et al.* [4] now show that BRCA2 is required to stabilize blocked DNA-replication forks and prevent the formation of double-strand breaks (DSBs) in the DNA. This has important implications for the manner in which loss of BRCA2 leads to chromosomal instability.

Lomonosov *et al.* used murine embryo fibroblasts with a homozygous truncation in BRCA2 and compared them with cells containing wild-type (WT) BRCA2, derived from the same genetic background. These cell lines were treated with hydroxyurea (HU), which mimics genome-wide replication blocks.

The authors examined a region adjacent to the origin of bidirectional replication within the rDNA locus. DNA replication intermediates were analyzed by 2D gel electrophoresis and Southern blotted. This produced an arc of Y-shaped DNA-replication intermediates in both cell lines. However, after HU treatment the arcs from the cells containing truncated BRCA2 rapidly reduced and disappeared, indicating that the DNA intermediates from this cell line are less stable. It was shown that the disappearance of Y-shaped intermediates is not due to DNA degradation or reduced sensitivity to HU.

Another explanation for the loss of Y-shaped intermediates is processing to produce DSBs. The authors showed that loss-of-function of BRCA2 reduces the stability of DNA replication intermediates by allowing them to be processed into DSBs and that BRCA2 functions downstream or in addition to Chk2.

These results suggest how BRCA2 mutations can lead to chromosomal rearrangements if the DSBs are randomly rejoined. This process is likely to be similar for many other genes that cause chromosomal instability disorders, which could also function in response to stalled DNA replication.

4 Lomonosov, M. *et al.* (2003) Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein. *Genes Dev.* 17, 3017–3022

Christian G. Noble
cnoble@nimr.mrc.ac.uk

Targets and Mechanisms

Understanding *CFTR* gene regulation

Gene expression and regulation of the cystic fibrosis transmembrane conductance regulator (CFTR) is not well known and most elements that control its expression have not been identified. CFTR is a chloride channel that belongs to the ABC transporter superfamily. The expression pattern is tightly controlled as CFTR proteins are targeted to the apical face of the cell and mainly found in lung, pancreas, colon and secretary glands.

Dysfunction and disregulation of CFTR are at the origin of cystic fibrosis. The precise mechanisms underlying CFTR spatial and temporal expression in different cell types remains to be understood. Mouchel *et al.* [6] now identify hepatic nuclear factor-α (HNF1α) as the first tissue-specific transcription factor involved in CFTR regulation.

Analysis of DNase1 footprinting fragments of several introns revealed the putative binding site for HNF1α within the CFTR gene. The authors also found via semi-quantitative RT-PCR that the expression of CFTR correlates to the expression of HNF1α in colon carcinoma cells (Caco2). HNFα and CFTR are co-expressed in cells that endogenously produced CFTR and, moreover, overexpression of HNFα increased CFTR expression levels.

Furthermore, the simultaneous expression of an HNFα antisense ribozyme in pancreatic Capan cells reduces the mRNA level of CFTR but seems to have no effect on the Caco2 cell, where the HNFα protein levels are more robust. Examining the expression of CFTR RNA levels from small intestine cells in HNFα knockout and wildtype mice shows that the ratio of CFTR:rRNA is significantly lower in knockout mice.

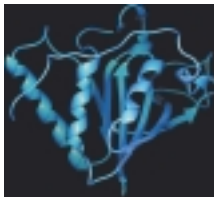
Although HNFα is involved in regulating the *CFTR* gene, HNFα is not sufficient to induce *CFTR* gene expression in cells that do not endogenously produce it. CFTR regulation, although different from one cell type to another, probably involves similar transcription factors and mechanisms.

6 Mouchel , N. (2003) HNF1alpha is involved in tissue-specific regulation of *CFTR* gene expression. *Biochem. J.* DOI:10.1042/BJ20031157 (E-publication ahead of print; http://www.biochemj.org/)

Muriel Laine
lainem@mail.rockefeller.edu

Miscellaneous

Protein-conducting channel: a monomer of the SecY complex



The protein-conducting channel (PCC, SecY/Sec61 complex) forms the translocation pore for protein secretion and membrane protein insertion. From previous electron microscopy structures it seemed clear that the PCC is an oligomer. Now, much to our surprise, the X-ray structure of the PCC presents a monomer of the SecY complex and there is no evidence for a large pore [7].

The X-ray structure of the translocation channel from the archaeon *Methanococcus jannaschii* was determined at 3.2 Å resolution. The structure suggests that the channel is formed by a monomer of the SecY complex comprising three subunits (Sec α,β,γ). The β- and γ-subunit contain only one transmembrane segment. The α-subunit consists of 10 transmembrane helices organized in two halves forming a clamp. The two halves (TM1-5, TM6-10) are related by a twofold symmetry. The clamp is believed to open between the TM2 and TM7, which would be the lateral gate for the release of membrane proteins from the channel. This is supported by cross-link studies.

The proposed channel is closed by a plug formed by a short helix (TM2a), which extends to the middle of the channel. This plug might move away during the translocation across the membrane. However, even when the plug is moved out, the channel would still be sealed by a pore ring formed by six conserved hydrophobic residues. Therefore, the membrane barrier is maintained during translocation.

7 van den Berg, B. *et al.* (2004) X-ray structure of a protein-conducting channel. *Nature* 427, 36–44

Irmi Sinning
Irmi.sinning@bzh.uni-heidelberg.de
Valérie Panneels
valerie.panneels@bzh.uni-heidelberg.de

Grant me a wish...

Science Info (http://www.escienceinfo.com) have announced the publication of an eNewsletter, the goal of which is to keep scientists informed of the latest information regarding grant and award opportunities in Life Sciences.

Contents of the first eNewsletter included: The Ellison Medical Foundation grant; The Drugs for Neglected Diseases Initiative Organisation; Bristol Myers Squibb Foundation grant; Pfizer Viagra Research Grants; American Cancer Society Grants; American Parkinson Disease Association, as well as useful websites.

To Subscribe for future issues, please visit http://www.escienceinfo.com/grant.htm.

Business

Collaborations

Bayer and Galapagos in target discovery collaboration

Galapagos Genomics (http://www.galapagosgenomics.com) have announced a target discovery collaboration with Bayer Healthcare (http://www.bayer.com) to discovery and validate novel drug targets. Galapagos will use its target discovery collections SilenceSelect™ and FleXSelect™.

Onno van de Stolpe, Chief Executive Officer of Galapagos, said: 'This partnership again underlines the competitive edge of our company in combining our SilenceSelect and FleXSelect libraries with high throughput disease biology.'

SilenceSelect is a collection of adenoviruses with siRNA based knockdown sequences targeting >4000 human druggable genes; FleXSelect is the mirror collection of adenoviruses with full-length genes from the druggable gene classes.

Galapagos is a drug discovery company focussed on the identification and validation of disease modifying drug targets by functional screening in human disease models, whose research activities encompass programs in osteoporosis, rheumatoid arthritis and Alzheimer's disease.

Epigenomics and Wyeth collaborate to identify drug response markers

Epigenomics (http://www.epigenomics.com) have announced an initial collaboration with Wyeth Pharmaceuticals (http://www.wyeth.com). This will involve the analysis, by Epigenomics, of DNA methylation biomarkers – in a murine xenograft model – that change after administration of an anti-cancer compound from Wyeth.

DNA methylation is a natural switch that controls gene expression giving rise to distinct patterns in cells. Such biomarkers could be further developed into drug response markers, predicting those patients who will benefit from drugs and those who will not.

Christina Dahlstroem, Vice President Product Development for Epigenomics' Pharma Technology business unit, said: 'DNA methylation analysis could be the detection method of choice for markers distinguishing between responders and non-responders to oncology drugs.'

Epigenomics is committed to personalizing medicine in cancer and other complex diseases by developing novel diagnostic and pharmacodiagnostic products.

Business was written by *Joanne Clough*

People

Appointments

Immtech names new CSO

Lawrence A. Potempa has been appointed as Chief Scientific Officer for Immtech International (http://www.immtech.biz), a pharmaceutical company dedicated to the commercialization of oral treatments for infectious diseases, fungal infections, tropical diseases and pneumonia.

Potempa has more than 25 years' experience in medical research and drug development in the areas of microbiology, biochemistry and immunology. His research focused on the development of substances that boost the human immune system and strengthen body defences against infection and cancer. This new role will involve working with Immtech's Scientific Consortium, which consists of 12 university research groups, working to accelerate drug discoveries into human clinical trials.

Potempa commented that: 'I am very happy to see increased foundation support toward solving global health problems. Increases in international travel and population migration cause new and challenging health threats that our drug candidates are in studies to address.' The company's drug development pipeline currently contains compounds that are claimed to be effective against a diverse range of such infections.

New appointments at CLOSURE Medical

CLOSURE Medical Corporation (http://www.closuremed.com), a leader in biomaterial-based medical devices, has announced the appointment of three new Vice Presidents. J. Michael Hoban, Bruce J. Krattenmaker and Gabe N. Szabo have taken the positions of Vice Presidents of Human Resources, Regulatory, Clinical and Quality Assurance and New Product Development, respectively.

CLOSURE President and CEO, Daniel A. Pelak remarked: 'During 2003, we have been executing a strategic plan that requires the continued development of multiple products and expansion into new markets. As a part of this plan, we have expanded our management team and realigned our organization with a view towards achieving our strategic objectives.'

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