*in silico* screening method does not comply with the patentability requirements.

The report generated from the collaboration of the three patent offices is important to scientists and researchers that will file computer-related patent applications, and provides guidance to biotechnology patent practitioners on how to apply their patent law principles to inventions in the emerging field of 3D protein structural analysis. Understanding how these patent offices will analyze these types of inventions clearly establishes the pitfalls that should be avoided and is crucial to formulating winning strategies for the prosecution and defense of patent infringements and patents during litigation.

1 Shimbo, I. *et al.* (2004) Patent protection for protein structure analysis. *Nature Biotech.* 22, 109–112

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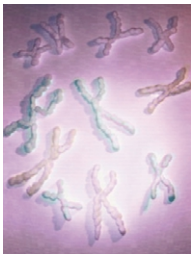
# Biology

## Molecular Biology

### Tethering chromatin



The 3D arrangement of chromatin in the cell nucleus is non-random and plays a major role in regulating genomic processes such as gene expression, replication and recombination. This structural organisation is thought to be achieved, in part, by attachment of chromatin to a proteinaceous scaffold, known as the nuclear matrix. Matrix attachment regions (MARs) are normally A-T rich DNA elements of roughly 100-1000bp; however, they display considerable sequence heterogeneity. Consequently, they are generally identified biochemically either as: DNA capable of binding isolated nuclear matrix preparations; or DNA fragments remaining matrix-attached after excision of intervening DNA loops by topoisomerase II (matrix-associated enzyme). Attached and non-attached DNA can also be visualised cytologically by performing nuclear 'halo' preparations (using high salt or LIS extraction). Staining these with DAPI (DNA stain) reveals matrix-attached chromatin as a bright, DAPI-dense, core and the non-attached, emanating loops as a diffusely stained halo. Surprisingly, the assumption that biochemically identified MARs and loops correspond to cytologically identified DAPI-dense and -diffuse regions, had not been formally tested - until now.

Sergey Razin's group have now mapped loops and MARs at the human dystrophin locus by topoisomerase-mediated excision, demonstrating, for the first time, that these biochemically identified elements do indeed correlate to the visible loops and MARs of halo preparations [1]. The exact nature and indeed existence of the nuclear matrix, is currently under debate, since the model of a rigidly fixed structure is not in agreement with recent kinetic studies of nuclear protein movements. Having now established that biochemically-defined and cytologically-defined MARs are one and the same it will be interesting to determine the dynamics of matrix association at these sites.

1 Iarovaia, O.V. *et al.* (2004) Visualization of individual DNA loops and a map of loop domains in the human dystrophin gene. *Nucleic Acids Res.* 32, 2079–2086

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## Targets and Mechanisms

### Folate and pterin metabolism: a new target

The development of *Leishmania* resistant to antimony-containing-drugs requires the rapid identification of new drug targets. El Fadili, A. *et al.* now report that the folate and pterin metabolism could provide new drug targets. Their study is based on the observations that the protozoan parasite *Leishmania* is a folate and pterin auxotroph and that the folate and pterins metabolisms are interconnected [2].

To overcome the pterins auxotrophy, parasites have developed a pterin transport mechanism through the BT1 receptor and reduction of the derivates into an active molecule by a pterin reductase (PTR1). Therefore, in order to futher understand the interconnection between folate and pterin metabolism, the authors generated BT1–PTR1 null mutant. The null mutant was obtained by homologous recombination and loss of heterozygocity by increasing the concentration of the selective drug. The mutant grew in a folate-rich medium (15 μM) but, surprisingly, also in a low-folate medium (20 nM). Because *Leishmania* cell resistance to methotexate (MTX) could be obtained by gene deletion of folate transporters or by transfection with PTR1 or BT1, the authors analyzed the MTX resistance of the BT1–PTR1 mutant. It was found to be 200-times more sensitive to MTX, compared with wild-type cells in SDM-79 medium. The roles of BT1 and PRT1 were confirmed by the finding that transfectants overexpressing these proteins became resistant to MTX, at least in the folate-rich medium.

In a next step the authors demonstrated that the parasite responds to gene inactivation by metabolic modification. They first observed that BT1–PTR1 null mutant resulted, for an non-elucidated reason, in the deletion a folate transporter gene. Second, because of an increase of folylpolyglutamatesynthetase (FPGS) activity, the polyglutamylation of folate and MTX was increased. Intriguingly, the authors remark that reduced polyglutamylation of MTX by inactivation of a copy of *FPGS*, for example, can lead to MTX resistance.

In order to identify new genes associated with MTX resistance, El Fadiji *et al.* generated two MTX-resistant clones from

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## Microbiology

### Diphtheria and vaccination

Diphtheria remains a serious bacterial disease throughout much of the world. Most life-threatening cases occur in unvaccinated or inadequately immunized persons. The phenomenon of decreasing immunity in adults continues to be observed in many European countries. The large outbreaks of diphtheria recorded in the 1990s throughout Russia and the newly independent states (NIS) of the former Soviet Union could well be attributed to inadequate population immunity. A booster dose of diphtheria toxoid in adults can produce productive antibody levels in a large percentage of subjects, reinforcing the efficacy of repeated revaccination against diphtheria for continuous protection.

Bissumnhar *et al.* now evaluate the possibility of considering patients as donors for the production of human immunoglobulin in emergency situations, where the availability of anti-diphtheria immunoglobulin (DAT) of either equine or human origin can be limited [7]. The selection criteria for potential donors in this study included high antibody titre and absence of other specific disease markers. The arbitrary cut-off point of 3.0 IU/ml was based on previously published work. The antibody titres in treated and untreated convalescent patients during an epidemic were well within this cut-off point. It was concluded that, in an emergency situation, it was possible to select donors among convalescent patients.

The authors also evaluated the effect of booster immunization with diphtheria toxoid and persistence of anti-diphtheria toxin antibodies in these patients. The pre-vaccination antibody titres in the subjects were varied, but 50% had adequate antibody titres and were selected as immediate potential donors. After booster vaccination, 70% had adequate antibody titre levels. This demonstrates the importance of selecting convalescent patients in a long-term policy to prepare an appropriate stock of anti-diphtheria toxin immunoglobulin. Finally, the authors concluded it necessary to have a group of adult donors immunized periodically with the diphtheria toxoid vaccine.

7 Bissumbhar B. *et al.* (2004) Evaluation of diphtheria convalescent patients to serve as donors for the production of anti-diphtheria immunoglobulin preparations. *Vaccine* 22, 1886–1891

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### Drug resistance and horizontal gene transfer

Some strains of enterotoxigenic *Escherichia coli* (ETEC) express a type of pili belonging to the CS1 family, and the genes for these pili are present on a large plasmid termed pCoo. A study by Froehlich *et al.* now shows that the pCoo plasmid is a member of the IncI1 incompatibility group and shows limited similarities in organization and DNA homology to the drug resistance plasmid R64 [8].

Although pCoo is not self-transmissible, the authors show that it can be transferred in the presence of R64. The pCoo plasmid derivatives that were recovered from the transconjugants differed in structure from the original pCoo plasmid as a result of recombination events with R64 that allowed the transfer to occur. Single- and double-crossover events between pCoo and R64 were shown to lead to the formation of the new, transferred pCoo derivatives.

The results demonstrate how two related plasmids, one encoding pilus genes used for adherence and one encoding resistance to antibiotics, can interact to facilitate horizontal transfer among bacterial hosts. Observations such as these show how new pathogenic and drug resistant strains can evolve via horizontal transfer.

8 Froehlich, B., E. Holtzapple, T.D. Read, and J.R. Scott. 2004. Horizontal transfer of CS1 pilin genes of enterotoxigenic *Esherichia coli*. *J. Bacteriol.* 186(10):3230-3237.

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the BT1–PTR1 null mutant. These two clones showed a decrease in folate and MTX uptake. Moreover, they observed a complete absence of MTX polyglutamylation in the MTX 500.3 clone. This absence was not due to a loss of the FPGS activity and the mechanism involved in these changes in the distribution of polyglutamates has yet to be elucidated.

The authors have demonstrated that *Leishmania* are able to grow in a medium greatly diminished in folate and pterin, leading to drastic changes in MTX polyglutamylation and concluded, because of its unique feature, that the folate and pterin metabolism could provide new drug-target.

2 El Fadili, A. *et al.* (2004) Inactivation of the *Leishmania tarentolae* pterin transporter (BT1) and reductase (PTR1) genes leads to viable parasites with changes in folate metabolism and hypersensitivity to the antifolate methotrexate. *J. Biol. Chem.* 279, 18575–18582

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### NF-κB inhibitors: more harm than good?

Despite sporadic evidence for a proapoptotic function, NF-κB has been widely regarded as a transcription factor whose activation protects cells from apoptosis. This notion was seriously challenged by a publication in *Nature* from the group of Karen Vousden claiming that the tumour suppressor protein p53 requires NF-κB for apoptosis induction [3]. This result has been controversial and two years later, Inder Verma and co-workers reported a contradictory finding, that is, that NF-κB activation decreased p53 stabilization and cell death in cells treated with the DNA-damaging agent doxorubicin [4].

Two recent papers currently in press with *The Journal of Biological Chemistry* might eventually lead to a resolution of this controversy. In the first [5], Warner Greene's group reports a novel mechanism of genotoxic stress-induced, p53-mediated NF-κB activation bypassing the canonical pathway (IκB kinase activation followed by IκB phosphorylation and consequent degradation). In this novel pathway, the p65 subunit of the transcription factor is phosphorylated by RSK1, resulting in prolonged NF-κB activity owing to

decreased nuclear export. These results are in line with some of the findings of Vousden's group, but leave the question open as to whether NF-κB activation via this pathway indeed increases p53-stimulated apoptosis or decreases it, as suggested by the results of Verma and colleagues.

In the second article [6], Fujioka *et al.* describe p53 stabilization as a novel mechanism for a proapoptotic function of NF-κB. However, this mechanism was found not for DNA-damaging agent but protein synthesis inhibitor doxycycline-induced p53 activation. This compound is believed to inhibit the expression of mitochondrial electron transport chain proteins resulting in the release of superoxide which activates NF-κB. Doxocycline-induced p53 activation and apoptosis was found to depend on the canonical NF-κB pathway, but activation of the transcription factor by this drug does not require p53. Instead, p53 is stabilized to exert its proapoptotic effect, apparently by NF-κB-dependent downregulation of the E3 ubiquitin ligase HDM2 level.

These findings have serious implications for the use of NF-κB inhibitors as adjuncts in anticancer chemotherapy. Although the majority of human tumours lack functional p53, for those that have retained it, the combination of NF-κB inhibitors currently in development with some standard chemotherapeutic drugs might result in increased tumour cell survival rather than death. Further investigation of this important issue will be required before a clear rationale for the inhibition of NF-κB as strategy in anticancer combination chemotherapy can be developed.


3 Ryan, K.M. *et al.* (2000) Cancer: pinning a change on p53. *Nature* 404, 892-897

4 Tergaonkar, V. *et al.* (2002) p53 stabilization is decreased upon NFkappaB activation: a role for NFkappaB in acquisition of resistance to chemotherapy. *Cancer Cell* 1, 493-503

5 Bohuslav, J. *et al.* (2004) p53 Induces NF-kappa B activation by an Ikappa B kinase-independent mechanism involving RSK1 phosphorylation of p65. *J. Biol. Chem.* DOI: 10.1074/jbc.M313509200 (E-publication ahead of print; http://www.jbc.org)

6 Fujioka, S. *et al.* (2004) Stabilization of p53: a novel mechanism for proapoptotic function of NF-kappa B. *J. Biol. Chem.* DOI: 10.1074/jbc.M313435200 (E-publication ahead of print; http://www.jbc.org)


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# Business

## Collaborations

### Inpharmatica and Procter & Gamble

Inpharmatica (http://www.inpharmatica.com) have announced a discovery collaboration with Procter & Gamble Pharmaceuticals (P&GP; http://www.pgpharm.com). Under the terms of the agreement, P&GP will fund a research program at Inpharmatica and pay additional license fees to use discoveries arising from the collaboration.

The goal of the collaboration will be to develop robust computational structure models and identify chemotypes for families of human G-protein coupled receptors (GPCRs). Inpharmatica will apply proprietary elements of its drug discovery platform PharmaCarta™. Specifically, the Chematica™ component will provide 3D homology modelling, drug binding-site identification and mapping techniques, and proprietary databases will help to identify tractable chemical hits and GPCR family chemotypes.

Malcolm Weir, Chief Executive Officer at Inpharmatica commented, '…this agreement constitutes a very exciting program for Inpharmatica. It will allow the company to use its cutting edge technologies to address the problem of identifying new drugs targeted to members of one of the most important families of drug targets.'

P&GP's Director of Chemistry and Discovery Technologies, Joseph Gardner, said: 'We are always looking for ways to increase productivity in drug discovery, and collaboration with Inpharmatica will move us towards this goal… We expect our joint efforts will bring benefits to both sides.'

### ParAllele BioScience announce two collaborations

ParAllele BioScience (http://www.parallelebio.com) has announced two collaborations; an SNP research agreement with Merck (http://www.merck.com), and the early access deal for the company's commercial SNP genotyping solution with the National Cancer Institute (NCI; http://www.nci.nih.gov).

In the Merck collaboration, ParAllele will use both its SNP discovery and SNP genotyping technologies to discover genetic variations that could impact the disease susceptibility, prognosis or response to therapy of an individual, in order to pursue improved drug targets.

ParAllele is also launching an 'out-of-the-box' solution for highly multiplexed SNP genotyping, thus enabling researchers to obtain valuable results at the bench top. The NCI has signed as an early access customer for the company's MegAllele™ SNP genotyping kits.

## Funding

### UCLA Lab2Market Investment Fund

University California, Los Angeles (UCLA; http://www.ucla.edu), has established an investment fund aimed at accelerating the conversion of laboratory discoveries into commercial uses. The UCLA Lab2Market Investment Fund provides up to USD$25,000 to individual faculty whose research shows promise in the marketplace but who lack funding for additional experiments required to demonstrate commercial viability.

Associate Vice Chancellor for Research Andrew Neighbour, who directs UCLA's Office of Intellectual Property Administration, said: 'By providing funding to faculty for research to aid in product development, the UCLA Lab2Market Investment Fund facilitates the transfer of technology to the marketplace and eliminates a significant barrier in the development of marketable ideas and products.'

The first recipient of a grant from the Investment Fund is Farhad Parhami, an Associate Professor of Medicine at the David Geffen School of Medicine, whose research has shown that certain oxysterols stimulate bone-forming cells that could help in the future treatment of osteoporosis. Parhami hopes to form a company to develop medications based on his research but investors first want to see positive results in animal models, rather than bone cells. Grants for this further research might become available but could take more than a year for application and approval, a timeframe that is not conducive to entrepreneurial product development, which is where the Lab2Market Investment Fund comes in.

The USD$300,000 Fund was established with equal contributions from three Californian venture capital firms: Cycad Group of Santa Barbara, Draper Fisher Jurvetson of Menlo Park and Zone ventures of Los Angeles.

Business was written by *Joanne Clough*

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