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Monitor

***Monitor* provides an insight into the latest developments in the pharmaceutical and biotechnology industries. *Chemistry* examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while *Profiles* provides commentaries on promising lines of research, new molecular targets and technologies. *Biology* reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. *Business* reports on the latest patents and collaborations, and *People* provides information on the most recent personnel changes within the drug discovery industry.**

Chemistry

Patents

Patentability issues of 3D protein structures

On 2 December 2002, the US Patent and Trademark Office (USPTO) announced the publication of a report, which was prepared and issued in conjunction with the European Patent Office (EPO) and Japan Patent Office (JPO), that provides guidance for the filing of patent applications concerning 3D protein structural data and pharmacophores, with significant implications for the biotechnology industry. In a recent publication, Shimbo and colleagues [1] gave an excellent summary of this joint patent office report.

The majority of bioinformatics innovations involve the application of computer-implemented protocols or software to collecting and/or processing biological data. These bioinformatics inventions fall within the general category of computer-related inventions that must be a 'useful' process, machine, article of manufacture or composition of matter (i.e. the process must have a practical application). The purpose of this requirement is to limit patent protection to inventions that have a level of 'real world' value, as opposed to subject matter that represents nothing more than an idea or concept, or is simply a starting point for future investigation or research. For example, products of nature, such as vitamins and minerals, are non-patentable in their naturally occurring state. However, if a natural product is new, useful and has been altered by humans in some way (e.g. purification, concentration, combination or isolation of the product), it could then be patentable.

Computer modeling and screening algorithms that are applied to biological data can now characterize a protein by its complex 3D structure, thus aiding in the design of potential pharmaceutical products. By using such computational methods, the drug discovery process can be accelerated and the associated costs reduced. With the elucidation of more 3D structures of chemicals and proteins, the USPTO, EPO and JPO expect an increase in the number of applications filed relating to this 3D structural information. With respect to patentability in general, these three patent offices have similar requirements (i.e. novelty of the product, industrial applicability, description and enablement of the disclosure and clarity of claims). There were four general case study categories cited in the report: (i) claims to the underlying protein structural data; (ii) claims for the proteins, polypeptides and protein domains that are defined by the data; (iii) *in silico* screening methods; and (iv) pharmacophores and pharmacophore-defined compounds. For clarification, a pharmacophore is defined by the spatial arrangement of the chemical elements of those functional groups that have been determined to be essential for the biological activity of a drug.

Not surprisingly, the USPTO, EPO and JPO agreed that claims relating to protein structural data, directed to mere data or data representations, were non-patentable subject matter. Such claims included 3D structural information, data arrays, data compilations of atomic coordinates of proteins or pharmacophores, computer-readable media containing such data and computer models of proteins that are based on such data. In cases where a 3D protein structure was already known, the claim for an isolated and purified molecule that conforms to a binding pocket of the protein, as defined by the structural coordinates, did not comply with any of the patentability requirements.

A claim for pharmacophore-defined compounds, or their salts, that lacks demonstrated utility of synthesized examples does not meet the requirements of enablement, support, clarity and/or written description. A pharmacophore is an abstract idea and not a specific compound or article of manufacture.

With respect to *in silico* screening methods, a claim for compounds identified by *in silico* screening methods alone is non-patentable subject matter. Furthermore, in a case where the description gives no examples of compounds that have been identified using the atomic coordinates of the protein, and the difference between prior art and the claimed invention as a whole is limited to atomic coordinates that are stored on a machine, the claim for an

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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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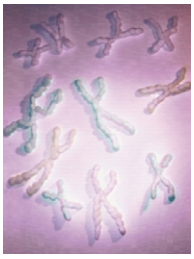
Biology

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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