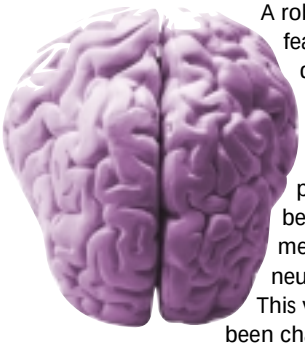
Biology

Neuroscience

Alzheimer's disease: depletion of calcium-dependent proteins explains cognitive deficits



A robust pathological feature of Alzheimer's disease (AD) is the appearance of neuritic plaques that contain the Aβ peptide, which is believed to lead to memory deficits and neurodegeneration. This view, however, has been challenged by clinical and preclinical evidence suggesting that memory loss can occur without the presence of these plaques. Recent research [1] now offers some clues as to how this might occur.

Palop *et al.* measured the levels of the calcium-binding protein, Calbindin-D28K (CB), as well as the product of the calcium-dependent immediate early gene, c-*fos* in transgenic mice expressing a mutant human APP gene. CB and c-Fos levels were significantly reduced in the granule cells of the dentate gyrus of the transgenic mice and the changes were not due to neuronal loss. Similar reduction was observed in brain tissues obtained from deceased AD patients. The reduction of CB in transgenic mice was age-dependent but did not correlate with the amount of Aβ plaques or the level of APP overexpression. Importantly, there is also a strong correlation between CB levels and spatial learning performance of transgenic mice in a hippocampus- dependent task.

These results suggest that AD-related neuronal deficits are caused by small nonfibrous Aβ assemblies that can remain undetected anatomically but might influence synaptic function, and thus stimulus processing, before the appearance of plaques.

The results have two important implications. First, the investigators argue that biochemical or radiological measures of CB levels could serve as a surrogate marker upon which AD detection and treatment might be based in the clinic. Second, as CB has been shown to protect against Aβ-induced toxicity, this protein, or other components along its biochemical pathway, might represent good targets for pharmaceutical drug development.

1 Palop, J.J. *et al.* (2003) Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9572–9577

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The painful truth: the P2X class of ATP receptors

ATP is associated with transmission of nociceptive (pain) information within dorsal root ganglion (DRG) neurons and the spinal cord. Topical application of ATP to the skin elicits a painful sensation at the site of application.

The P_2X class of receptor molecules for ATP, P_2X_3 and $P_2X_{2/3}$, are ligand-gated ion channels that are excitatory to nociceptive neurons and are preferentially localized to the central and peripheral endings of sensory afferent DRG neurons, implicating them directly in nociceptive processing. The distribution and functional contribution of P_2X_3 and $P_2X_{2/3}$ receptors identifies them as potentially valuable targets for specific targeting agents designed to interfere with pain sensation.

A recent paper [2] has characterized the pharmacological selectivity and activity kinetics, as well as the anti-nociceptive behavioral effects, of a novel and selective non-nucleotide $P_2X_3/P_2X_{2/3}$ receptor antagonist, A317491. Developed by Abbott Laboratories (http://www.abbott.com), this compound has been shown to reduce pain-related behaviours commonly associated with acute peripheral inflammation and chronic nerve injury. In a variety of models, rats receiving A317491 demonstrated dose-related decreases in pain-related behaviours. A317491 also reduced the effectiveness of the P_2X receptor agonist α,β-meATP, demonstrating high receptor selectivity.

The regional specificity and distribution of the P_2X_3 and $P_2X_{2/3}$ receptor subtypes on nociceptive DRG neurons points to a putative role of these receptors in the signaling of nociceptive messages from the periphery to the spinal cord and brain. This paper suggests that, through the use of novel and specific antagonists to these receptors, such as A317491, P_2X receptor targeting could be useful in reducing acute or chronic types of peripherally-generated pain in human subjects.

2 McGaraughty, S. *et al.* (2003) Effects of A-317491, a novel and selective $P_2X_3/P_2X_{2/3}$ receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration. *Brit. J. Pharmacol.* 140, 1–8

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Un-seizing the gain in pain with novel antiepileptic agents

Sodium channels help to determine how neurons fire, therefore, over-activation or misexpression of sodium channels can contribute to the generation of pathologies, such as abnormal pain syndromes or epilepsy.

Multiple similarities exist between the high-frequency neuronal discharging observed in neuropathic pain and epilepsy. The anticonvulsant class of sodium-channel blockers is effective in suppressing high-frequency discharges in both of these conditions.

Originally developed for potential use in epilepsy, a new α-aminoamide termed – NW1029 – possesses potent sodium-channel blocking properties. It is an orally active, voltage and frequency-dependent neuronal sodium channel blocker with demonstrated efficacy in rat DRG sensory neurons. Veneroni *et al.* [3] presented findings demonstrating that NW1029 has a potent and long-lasting anti-nociceptive activity in several models of abnormal pain. After induction of peripheral inflammation or nerve injury, NW1029 was effective, in a dose-dependent manner, in reducing mechanical allodynia (where a normally non-noxious stimulus becomes noxious), apparently without neurological impairment. In acute-pain models, and in unaffected paws, NW1029 was ineffective in reducing pain-related behaviors.

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Recent investigations of the applicability of antiepileptic agents to other pathologies associated with abnormal, high-frequency neuronal discharging show great potential. The demonstration of the efficacy of NW1029 in abnormal, neuropathic pain syndromes, if proven to be more advantageous than commonly used anticonvulsant reference drugs that block voltage-gated sodium channels, such as phenytoin and lamotrigine, might hold promise for pain relief associated with post-operative, arthritic and neuropathic pain in the clinical setting.

3 Veneroni, O. *et al.* (2003) Anti-allodynic effect of NW-1029, a novel Na(+) channel blocker, in experimental animal models of inflammatory and neuropathic pain. *Pain* 102, 17–25

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Cancer

Another string to the aspirin bow

Aspirin is used clinically in the treatment and prevention of an ever-increasing number of disorders, ranging from cardiovascular disease and arthritis to cancer. Further investigations have suggested that the efficacy of aspirin might be attributed to the inhibition of cyclooxygenase-2, as demonstrated by more selective inhibitors of this enzyme. However, an alternative mechanism-of-action involving the angiogenic factor thymidine phosphorylase (TP) has recently been proposed. It relates to a potential anti-angiogenic effect of aspirin, which might bear relevance to it's activity, particularly in arthritis and cancer.

In a series of elegant experiments, Zui and Schwartz demonstrated that treatment of the THP-1 monocyte cell line with TNF-α, resulted in increased expression of thymidine phosphorylase (TP) mRNA, protein and enzyme activity [4]. More specifically, this increase in TP expression was mediated via the TNF-α receptor 2, as confirmed by the use of specific agonistic antibodies.

Further studies indicated that the increase in PMA-mediated TP expression was inhibited by aspirin but not by indomethacin, a non-steroidal anti-inflammatory and cyclooxygenase inhibitor, suggesting that the effect of aspirin on TP expression is cyclooxygenase independent. Moreover, further experiments suggested that this effect could be related to the ability of aspirin (but not indomethacin) to modulate NF-κB–DNA binding, as studies using an NF-κB inhibitor peptide confirmed a role for NF-κB in the TNF-α-induced TP expression. In addition, aspirin has been shown previously to inhibit NF-κB activation by preventing the degradation of IκB.

In summary, inhibition of TNF-α-induced TP-expression via NF-κB modulation might offer an alternative, non-cyclooxygenase mechanism-of-action for the anti-angiogenic effect of aspirin. This mechanism could explain the efficacy observed with aspirin in treatment and prevention of diseases, such as arthritis and cancer, with a known angiogenic component.

4 Zui, G.H. and Schwartz, E.L. (2003) Expression of the angiogenic factor thymidine phosphorylase in THP-1 monocytes: induction by autocrine tumour necrosis factor-alpha and inhibition by aspirin. *Mol. Pharmacol.* 64, 1251–1258
5 Kopp, E. and Ghosh, S. (1994) Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* 265, 956–959

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Genomics and Proteomics

RNAi used to reduce tyrosine hydroxylase levels in adult mice

The main problems of using knockout and transgenic mouse models in determining the molecular basis of disease are an inability to dissociate between developmental effects and effects in the adult, genetic compensation and a lack of spatial specificity. Using RNA interference, Hommel *et al.* [6] managed to generate a specific knockdown of the tyrosine hydroxylase (*Th*) gene (encoding the rate limiting enzyme in dopamine biosynthesis) in midbrain neurons of adult mice.

The authors used adeno-associated virus (AAV), which was engineered to express enhanced-GFP, enabling the identification of infected neurons. Injection of the experimental viruses into the substantia nigra (SN) of young mice resulted in a significant reduction in *Th* expression. Assaying the strength of expression of *Th* through immunostaining in the period following injection showed that, by day 12, the levels of the enzyme had decreased markedly, and that this decrease persisted until at least day 50.

To show the functional significance and specificity of these molecular measures, *Th* knockdown mice exhibited attenuated locomotor activity in a dopamine-dependent paradigm 16 days after injection in the VTA with the modified AAV. Also, mice injected bilaterally in the SN with the experimental virus exhibited motor deficits on the rotarod 17 days later, a finding that recapitulated the phenotype of neurotoxin-induced rodent models of Parkinson's disease and suggested that attenuation of dopamine biosynthesis might be sufficient to cause the motor deficits caused by degeneration of this brain region.

This study allows the effects of spatially restricted Th depletion to be determined in adult mice, thereby more accurately simulating human neurodegeneration and overcoming developmental difficulties. Moreover, the techniques used might be more generally applicable to create animal genetic disease models, and even in human gene therapy.

6 Hommel, J.D. *et al.* (2003) Local gene knockdown in the brain using viral-mediated RNA interference. *Nat. Med.* 9, 1539–1544

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Coordinated arrangement of linearly dispersed genes

The linear sequence of genomes exists in the 3D space of the cell nucleus, however, despite whole-genome sequence information, it is not clear how chromosomes and genes are arranged in this space. An attractive idea is that co-regulated genes are clustered to facilitate gene control and regulation. The only convincing evidence for such an arrangement has been the clustering of ribosomal genes in the nucleolus of eukaryotes. Now, evidence has emerged for clustering of transfer RNA genes in yeast [7].

Saccharomyces cerevisiae contains 274 t RNA genes, which are scattered across all of its chromosomes. Thompson *et al.* asked whether these linearly dispersed genes occupied random positions or whether they were clustered in the cell nucleus. They visualized genes from five of the 42 tRNA gene families by fluorescence *in situ* hybridization and found these genes to localize to a single large cluster near the nucleolus, which is consistent with the accumulation of tRNA-processing factors in or near the nucleolus in yeast and mammalian cells. The clustering of the tRNA genes was sensitive to the presence of RNA polymerase III, which suggests that formation of the transcription complex or transcription *per se* determines the genes' location.

So, what is the advantage of gene clustering? These observations on tRNA genes are important because they strengthen the concept of higher-order arrangement of genes in cell nucleus. In addition, they highlight how little we know about the fundamental principles of genome organization *in vivo* and how spatial genome organization contributes to gene control.

7 Thompson, M. *et al.* (2003) Nucleolar clustering of dispersed tRNA genes. *Science* 302, 1399–1401

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Drug Delivery

Microfabrication and new architectures in drug delivery

Liposomal or vesicular (niosomes) systems are frequently employed to deliver environmentally sensitive or toxic drugs or to target particular diseases. The use of niosomes is common for vaccine and drug delivery in addition to gene therapy and transfection. A recent study of extruded surfactant vesicles [8] showed polyoxyethylene ether surfactant (non-ionic) mixtures combined with cholesterol to create model niosome systems for investigation of drug encapsulation.

Nasseri and Florence [8] prepared niosomes by a well-known hand-shaking method. The ejection of niosomes from preformed rigid microcapillaries was followed by video camera imaging and light microscopy. The study reveals a series of findings in the form of detailed images: under normal circumstances in solution, polyhedral vesicles are formed from the aqueous polyoxyethylene ether:cholesterol surfactant mixtures, however, when the vesicles are forced through a micropipette of smaller dimensions than the average diameter of the preformed niosomes, they deform and give rise to fused products, which assume variations of shape centred around two unusual morphologies that the authors describe as 'whorls' and tubules. These tubular microengineered structures are elucidated by use of niosome encapsulated latex microspheres.

This work strikes an interesting balance of novel findings and tried-and-tested methods with conventional materials but new ideas of processing and many implications for adaptable drug-delivery systems. This raises the notion of mass manufacture of such soft condensed matter assemblies in terms of strategies for microengineering. The authors briefly discuss the future role of such material in polymerization as a means of microencapsulation of drug in more permanent drug-delivery vehicles and as models for biomineralization leading to further elucidation in this field.

8 Nasseri, B. and Florence, A.T. (2003) Microtubules formed by capillary extrusion and fusion of surfactant vesicles. *Int. J. Pharma.* 266, 91–98

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Immunology

The neutrophil comes home!

Neutrophils are released from the bone marrow and circulate for 7–10 h before removal by the reticulo-endoplasmic system. A concerted expression of two specific chemokine receptors (CXCR2 and CXCR4) provides fine control of neutrophil mobilization from, and return to, the bone marrow.

In vitro experiments [9] showed that CXR2 ligands activate human neutrophils, and this effect was attenuated by addition of a CXCR4 agonist, stromal-derived factor 1α or SDF-1α. However, SDF-1α was unable to promote cell activation by itself. These data led the authors to hypothesize that the system could be operative in *in vivo* settings. Therefore, moving to the experimental animal to challenge this hypothesis, the CXCR2 ligand KC synergised with the CXCR4 antagonist (termed AMD3100) in promoting blood neutrophilia, that is, mobilization from the bone marrow, following their systemic administration.

Superfusion of bone marrow femurs with the CXCR2 ligand KC or with the CXCR4 antagonist AMD3100 confirmed the existence of this dual tonic control on neutrophil mobilization, that is, CXCR4 activation (by SDF-1α) retains the neutrophil within the bone marrow; addition of a CXCR2 agonist would help trafficking into the blood. Another interesting aspect is that neutrophil senescence *in vitro* is not only associated with programmed cell death, but also characterized by CXCR4 upregulation on the cell surface, as detected by flow cytometry.

In conclusion, this study highlights a central role for two specific chemokine receptor and their ligands in controlling neutrophil trafficking in pathophysiology, and give emphasis on a process only in part known, that is the one of the mature senescent neutrophil going back to its primordial site, the bone marrow.

9 Martin, C. *et al.* (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* 19, 583–593

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Cryptococcus neoformans App1 protein helps fend off macrophages

Inositol-phosphoryl ceramide synthase 1 (Ipc1) is an essential enzyme in *Cryptococcus neoformans* and other yeast. Luberto *et al.* [10] previously showed that down-regulation of the *C. neoformans IPC1* gene through the use of a *GAL7* promoter has pleomorphic effects on virulence, including decreased melanin synthesis, decreased survival within macrophages, and decreased growth at pH 4. To further investigate the role of Ipc1 in *C. neoformans* virulence, the same group have identified a downstream effector gene (*APP1*) that is regulated by *IPC1*.

Luberto *et al*. [11] performed differential display RT-PCR on a *GAL7:IPC1* strain grown under repressing (glucose) and inducing (galactose) conditions. One of the induced bands was homologous to a *C. neoformans* protein that inhibits phagocytosis by alveolar macrophages. This sequence, designated *APP1*, was used to construct a Δ*app1* mutant that exhibited higher levels of phagocytosis compared with wild-type strains, but it did not exhibit the other phenotypes seen when *IPC1* is repressed. Experiments with purified App1 suggest that it acts by inhibiting attachment to a complement receptor.

The Δ*app1* mutant exhibited decreased virulence in A/Jcr mice (deficient in complement C5). In contrast, the mutant was more virulent than wild-type *C. neoformans* in Tgε26 mice (deficient in T cells and NK cells), possibly due to a 'Trojan horse' mechanism whereby increased phagocytosis leads to increased dissemination. Additional studies will be needed to determine the specific site of action of App1 and to understand how it is regulated *in vivo*.

10 Luberto, C. et al. (2001) Roles for inositol-phosphoryl ceramide synthase 1 (IPC1) in pathogenesis of *C. neoformans*. *Genes Dev.* 15, 201–212

11 Luberto, C. *et al.* (2003) Identification of App1 as a regulator of phagocytosis and virulence of *Cryptococcus neoformans*. *J. Clin. Invest.* 112, 1080–1094

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Miscellaneous

Bacterial antigens are not secreted by chance

The secretion of virulence-promoting proteins at appropriate locations and times during infection of host cells has emerged as a common virulence strategy of bacterial pathogens. For some Gram-negative bacteria, the presence of secretion systems can be used to distinguish pathogenic from non-pathogenic species. Whereas pathogenic Gram-negative bacteria have more than five distinct translocation pathways for shuttling proteins across their dual-membrane cell envelope, the mechanistic basis for controlled secretion of virulence proteins by Gram-positive bacteria and mycobacteria has thus far been a mystery.

Recent work by Stanley *et al.* [12] provides several clues towards solving this mystery by providing direct evidence that a new protein-secretion pathway contributes for virulence of *Mycobacterium tuberculosis* in mice, at least partially through its role in promoting secretion of the mycobacterial ESAT-6 and CFP-10 immunodominant antigens. Using a variation of the technique of signature-tagged transposon mutagenesis [13], Stanley *et al.* isolated *M. tuberculosis* mutants attenuated for growth during early stages of systemic mouse infection. Several of these mutations mapped to the *snm1*, *snm2*, and *snm4* genes, which surround the genes encoding ESAT-6 and CFP-10 on the *M. tuberculosis* chromosome.

How does secretion of the ESAT-6 and CFP-10 antigens promote *M. tuberculosis* virulence? These results suggest that ESAT-6 suppresses host innate immune responses by infected macrophages; including IL-12 expression, the release of TNF-α, and the production of anti-bacterial reactive nitrogen species. This work suggests that studies of auxiliary Gram-positive secretion systems and their substrates will uncover novel mechanisms for bacterial interference with host innate immunity.

12 Stanley, S.A. *et al.* (2003) Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13001–13006

13 Hensel, *et al.* (1995) Simultaneous identification of bacterial virulence genes by negative selection. *Science*, 269, 400–403

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Arginine kinase: a potential drug target for trypanosomes?

In a wide variety of invertebrates, phosphoarginine (PA) is the most important phosphagen. Arginine kinase (AK) has a role in maintaining ATP levels catalyzing the reversible transphosphorylation between ADP and phosphoarginine. By buffering the ATP levels, AK is therefore essential in cells with fluctuating energy requirements, for example, until glycolysis and oxidative phosphorylation are switched on.

A single-copy gene encoding a functional AK in *Trypanosoma cruzi* has previously been identified. In their recent study [14], Pereira *et al.* demonstrate that the overexpression of *T. cruzi* AK improves the capability of transfectant parasites to grow and survive when exposed to nutritional and pH stress conditions.

The gene encoding AK was cloned in the pTREX expression vector and transfected into epimastigote cells, giving pTREX–AK parasites. AK activity assays revealed that the AK-specific activity in pTREX–AK parasites remained constant along the growth curve, suggesting that the high expression level reached by this vector could mask the regulation process affecting this enzyme. No significant increase in the trypomastigotes population was observed in these media. However, the authors showed that the pTREX–AK population reached a higher cell density in the TAU-3AG medium. They attributed this difference to culture stresses caused by the medium.

These results and the fact that the arginine-specific activity and expression increase during the parasite growth led the authors to suggest that AK is a factor of an adaptive response to nutritional and pH stress conditions. In conclusion, this enzyme, which is totally different from

phosphagen kinases present in the mammalian hosts, could constitute a promising therapeutic target.

14 Pereira, C.A. *et al.* (2003) Arginine kinase overexpression improves *Trypanosoma cruzi* survival capability. *FEBS Lett.* 554, 201–205

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PI3K and chemotaxis: localized patches make the cell's eyes...

The connections between phosphatidylinositol 3-kinases (PI3K) and chemotaxis have been the subject of fevered research since it was shown that PIP3, their product, is localized to the nascent fronts of turning cells. However, there are still large holes in the story. One particular problem has been the need for some kind of inhibitor to stop uniform levels of chemoattractant from stimulating PI3K all over the cell surface. Such inhibitors have not been found despite widespread searches.

Postma *et al.* [15] now show that PI3K responses are localized differently to where researchers in the field had presumed. Using a GFP-tagged pleckstrin homology (PH) domain to follow the products of PI3K, they show that sudden stimulation of cells leads to a short-lived activation of PI3K on the cell surface, but within a minute or so this even stimulation resolves into discrete patches on the cell surface. Each patch covers about 10% of the cell, and there can be several (or none) on any cell. The patches are dynamic, with a lifetime of about a minute, and appear to be self-organizing. Formation of PI3K patches also correlates with the formation of new pseudopods.

These results suggest that PI3Ks respond to chemoattractants with a complex, self-organizing response, which is separate from the process of movement, but guides the formation of new protrusions. PI3K activity is not graded from the front to the back of the cell, but the patches presumably correlate with local chemoattractant concentrations, and thus guide the cell. A new model for PI3K stimulation should also break the deadlock in understanding the precise molecular details of how PI3K activity is regulated.

15 Postma, M. *et al.* (2003) Uniform cAMP stimulation of *Dictyostelium* cells induces localized patches of signal transduction and pseudopodia. *Mol. Biol. Cell* 14, 5019–5027

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People

Appointments

Julie Cherrington joins Phenomix Pty as President

Phenomix Pty (http://www.phenomixcorp.com), the Australian subsidiary of Phenomix, has appointed Julie Cherrington as President. Cherrington, who was formerly Vice President of preclinical research and exploratory development at SUGEN, will be responsible for advancing drug candidates towards preclinical development.

Prior to her time at SUGEN, Cherrington held several senior positions at Gilead Sciences, culminating in a position as Director of Virology.

Laura K. Shawver, President and CEO of Phenomix, said: 'Julie is an expert at handling the issues that emerge between late-stage preclinical development and the onset of clinical trials… We plan to make the most of her expertise in linking discovery and clinical development as we develop lead compounds for metabolic syndrome and immune disorders.'

Phenomix uses whole animal technology to rapidly discover and develop new treatments for disease, with programs in immune disease and metabolic syndrome.

Ardais appoints Donald B. Hawthorn as President and CEO

Donald B. Hawthorn has been appointed as President and Chief Executive Officer of Ardais (http://www.ardais.com), replacing Gregory D. Phelps. Hawthorn has held leadership positions in the healthcare industry for more than 20 years and has extensive experience in growing emerging companies.

Since 1999, Hawthorne has been a contract-operating partner to the healthcare venture capital community; formerly he was a Partner and Chief Financial Officer at Ampersand ventures.

William Mills, member of the Board of Directors at Ardais, said: 'The application of human disease to drug discovery – clinical genomics – is a rapidly emerging field. Ardais has already made a significant impact in this area… and we are excited about additional near-term business opportunities, which we are developing.'

Ardais is a privately held clinical genomics company dedicated to enhancing and accelerating biomedical research by the application of human disease as the discovery model in pharmaceutical R&D.

James Alexander named as Senior VP at Pozen

Pozen (http://www.pozen.com) have announced the appointment of W. James Alexander as Senior Vice President, Product Development. He will be responsible for leading the company's product development activities, including overseeing toxicology, clinical operations, biostatistics, data management and regulatory affairs.

John R. Plachetka, Chairman, President and CEO of Pozen, said: 'Jim's extensive

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