4 Dogruer, D. *et al.* (2004) 3-(4-Aminobutyn -1-yl)pyridines: binding at $\alpha_4\beta_2$ nicotinic cholinergic receptors. *Bioorg. Med. Chem. Lett.* 14, 523–526
5 Glennon, R.A. *et al.* (2000) Central nicotinic receptor ligands and pharmacophores. *Pharm. Acta Helv.* 74, 103–114
6 Lee, M. *et al.* (2002) A comparison of the binding of three series of nicotinic ligands. *Bioorg. Med. Chem. Lett.* 12, 1989–1992
7 Sheridan, R.P. *et al.* (1986) The ensemble approach to distance geometry: application to the nicotinic pharmacophore. *J. Med. Chem.* 29, 899–906
8 Tønder, J.E. *et al.* (2001) Agonists at the $\alpha_4\beta_2$ nicotinic acetylcholine receptors: structure–activity relationships and molecular modeling. *Curr. Med. Chem.* 8, 651–674

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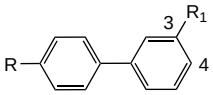
Small molecule inhibitors of the E1 helicase of human papillomavirus

Papillomaviruses are small DNA viruses that infect and replicate in the cutaneous or mucosal epithelia of human and other mammals. These viruses share a common genomic organization. However, the only encoded protein that has enzymatic activity is E1, a DNA helicase. High-throughput screening of a collection of Boehringer Ingelheim compounds was performed to measure the ATPase activity of recombinant human papillomavirus 6 (HPV6, the virus responsible for the majority of cases of genital warts) E1 helicase. The screening revealed **va** as a lead compound (IC_{50} of 2 μM), which exhibited the characteristics of a specific, reversible inhibitor able to interfere with the affinity of E1 helicase for ATP. Modifications of the lead compound showed that, although the sulfonylacetic acid moiety was not tolerant to modification, substitution at the 3 and 4 positions was possible. Thus, a parallel synthesis approach afforded several highly active compounds (e.g. **vb**, IC_{50} of 0.004 μM). However, these compounds were not active in a cell-based assay of HPV replication, probably as a result of the high polarity of the negatively charged sulfonylacetic acid group or the chemical instability of this group. The corresponding nitromethyl sulfone **vc**, (IC_{50} of 0.63 μM) was shown to be permeable in a cellular assay (Caco-2) and, even if it is not potent enough to show cell-based activity, can be considered a new lead compound [9].



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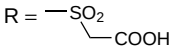

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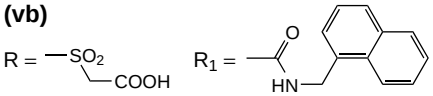

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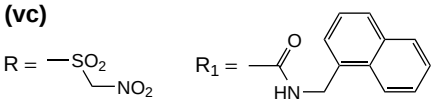

9 Faucher, A-M. *et al.* (2004) Discovery of small molecule inhibitors of the ATPase activity of Human Papillomavirus E1 Helicase. *J. Med. Chem.* 47, 18–21

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Biology

Microbiology

S. pyogenes vascular leakage can be inhibited by integrin antagonist

Streptococcus pyogenes can cause severe tissue damaging and systemic infections with high mortality. Hallmarks of systemic disease (toxic shock syndrome, STSS) are severe hypotension and multi-organ failure.

One of the most studied virulence factors of *S. pyogenes* is the cell wall-anchored M protein, which confers resistance against bacterial killing by neutrophils and binds several human proteins, including fibrinogen. M protein is initially anchored to the cell wall of the bacterium but can be released by proteinases, however, the pathophysiological consequences of this have not been investigated in detail.

Herwald *et al.* [1] show that proteinases secreted from human stimulated neutrophils release several fibrinogen-binding M protein fragments, which in turn trigger the neutrophils to aggregate and release the inflammatory mediator heparin-binding protein (HBP). The aggregation and activation of neutrophils was not directly mediated by M protein, but rather M protein–fibrinogen complexes.

Experiments using a β2 integrin peptide antagonist (Gly-Pro-Arg-Pro) showed that M protein–fibrinogen complexes activate neutrophils by β2 integrin crosslinking. Furthermore, intravenous injection of M protein into mice caused severe hemorrhagic lung lesions that could be reversed by co-injection of Gly-Pro-Arg-Pro. Finally, analysis of infected soft tissue from a patient with necrotizing fasciitis and STSS revealed that M protein is released from bacteria into the surrounding tissue and is co-localized with fibrinogen.

Overall, this study elegantly elucidates a series of events that might explain the rapid progression from localized *S. pyogenes* infection to severe systemic disease. In addition, the results obtained with the β2-integrin peptide antagonist could represent a much needed novel therapeutic strategy against STSS.

1 Herwald, H. (2004) M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. *Cell* 116, 367–379

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Genomics and Proteomics

Epigenetic silencing: neither the chicken nor the egg came first

In 'epigenetic silencing', cells shut down whole sectors of their genomes in a way that is stable over many cell divisions. This silencing involves covalent 'marking' of the DNA and histone protein components of the local chromatin, for example by methylation.

There has been much debate about whether the primary event in epigenetic silencing is histone methylation, with DNA methylation being a consequence of silencing or vice versa. In a recent study [2], Mutskov and Felsenfeld demonstrate that, in one model system, neither histone methylation nor DNA methylation is a primary event in the decision to silence, but rather they could be part of a later 'locking' mechanism.

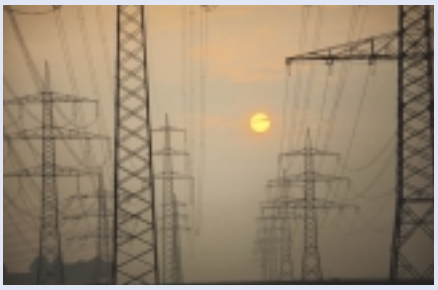
The authors studied the epigenetic silencing of stably integrated arrays of transgenes in chicken cells; they mapped each modification at different time points and compared the timing of its appearance with the timing of transcriptional extinction at the locus.

They found that methylation of DNA and of histone H3 at lysine 9, both previously thought to be possible triggers for silencing, occur several days after silencing is underway. The DNA and histone H3 lysine 9 marks could be a later event in the cascade, and might maintain or lock the silenced state in an irreversible way.

The authors point out that their study relates to the artificial system of integrated transgenes, and that the order of endogenous epigenetic cascades could be gene specific. Nevertheless, this study underlines the importance of studying timing as well as correlation in epigenetic silencing, and serves to illustrate how this could be done for specific endogenous genes.

Targets and Mechanisms

A map of the TNF-α–NF-κB regulatory network



In an almost gargantuan effort, scientists at Cellzome (http://www.cellzome.com) have performed large-scale mapping of the protein–protein interactions that form the regulatory network controlling NF-κB activation by TNF-α. Starting from 32 known and candidate components and using an integrated approach involving tandem affinity purification (TAP), LC–MS, network analysis and gene knockdown by RNA interference (RNAi), Bouwmeester *et al.* identified 131 interacting proteins, 80 of which were found to be novel, and a connections map of the network was generated [5].

Of the 80 novel interacting partners, 28 were put to the test by RNAi in an NF-κB-dependent reporter-gene assay for functional validation. These experiments led to the identification of 10 new modulators of TNF-α-induced NF-κB activation, two of which had no previous functional annotation: a novel member of the TRAF family (TRAF7) and a novel binding protein of the protein kinase TBK1 (TBK1BP). TRAF7 was identified by its association with TAP-tagged MEKK3 and this interaction was confirmed by co-precipitation of endogenous MEKK3. Furthermore, the MEKK3 interaction domain of TRAF7 was mapped and cytoplasmic co-localization of the proteins in vesicular structures, as well as phosphorylation and ubiquitination of TRAF7 induced by wild-type but not kinase-dead MEKK3, were observed. Moreover, overexpression of TRAF7 resulted in activation of NF-κB- or AP-1-dependent reporter-gene expression only when wild-type MEKK3 was co-expressed.

Based on these results, the authors propose that the relationship between TRAF7 and MEKK3 in TNF-α-induced NF-κB activation resembles that of TRAF6 with TAK1 in IL-1/Toll-like receptor-stimulated activation of the transcription factor. When integrated with computer models currently being developed [6], this map of the TNF-α/NF-κB signalling network, at the heart of many pathologies, could lead to improved identification and validation of potential drug targets.

5 Bouwmeester, T. *et al.* (2004) A physical and functional map of the human TNF-alpha/NF-kappaB signal transduction pathway. *Nat. Cell Biol.* 6, 97–105

6 Cho, K.H. *et al.* (2003) Investigations into the analysis and modeling of the TNF alpha-mediated NF-kappa B-signaling pathway. *Gen. Res.* 13, 2413–2422

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2 Mutskov, V. and Felsenfeld, G. (2004) Silencing of transgene transcription precedes methylation of promoter DNA and histone H3 lysine 9. *EMBO J.* 23, 138–149

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A different kind of messenger: sRNA labels send silencing complex to the right address

In recent years, a revival of interest in heterochromatin has revealed its secret life as being surprisingly dynamic and exquisitely specific. In fission yeast, heterochromatin (at the telomeres, centromeres and silent mating-type loci) silences, contains the Swi6 and Chp1 proteins, and is rich in methylation of lysine 9 on histone H3.

These modifications are necessary for heterochromatic silencing, but it was previously unclear how they got targeted to specific regions of chromosomes. Verdel *et al.* [3] now show that one kind of specificity is provided by small RNAs, transcribed from centromeric

Miscellaneous

Grey matter volumes and densities in long-term smokers

Magnetic resonance imaging (MRI) studies have revealed significant structural abnormalities in the brain that are associated with long-term smoking. Smoking is a risk factor for silent and symptomatic stroke, and studies have shown cigarette use is linked with ventricular and periventricular white matter irregularities.

A recent study [7] has documented associated abnormalities in grey matter volumes and densities, comparing 19 otherwise healthy smokers with 17 non-smoking controls. Smokers were defined as consuming 20+ cigarettes a day and met DSM-IV criteria for nicotine dependence. The groups were similar on average age, ethnicity, handedness, and depression and anxiety scores. The smokers consumed on average 26 cigarettes a day (20–40) and had a mean average 31 pack-year smoking history (range 9–70).

Exhaled carbon monoxide levels were measured as a rough index of recent cigarette smoking. MRI scans were performed at 7am with no smoking that morning. Hand-drawn regions of interest and the computer program voxel-based morphometry were used to assess group differences in regional grey matter volumes and densities, respectively. Brain regions of particular interest were the lateral prefrontal cortex (PFC), anterior cingulate cortex (ACC), ventral striatum and thalamus.

Smokers had smaller relative grey matter volumes and lower grey matter densities in the PFC bilaterally, smaller left dorsal ACC volumes and lower right cerebellar grey matter densities. Greater pack-year history was also found to be associated with lower PFC grey matter density. These brain areas have been linked with nicotine dependence, as well as cognitive and personality variables, such as the poorer working memory performance and higher impulsivity in smokers. These patterns could be linked to the effects of smoking, a predisposition towards smoking or related factors.

7 Brody, A.L. *et al.* (2004) Differences between smokers and non-smokers in regional gray matter volumes and densities. *Biol. Psych.* 55, 77–84

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heterochromatin, and that bring a specific complex to assemble heterochromatin at this region.

Two key proteins in the RNAi pathway are Dicer, which chops up double-stranded RNA precursors into short pieces, and Argonaute, which uses these pieces as a guide for sequence-specific recognition of the target molecule. Verdel *et al.* now address the question of how small RNAs are involved in heterochromatin assembly in fission yeast.

The authors purified a complex containing Chp1, a protein of unknown function (Tas3), and the Argonaute protein together with small RNAs that match centromeric DNA sequences. This combination of proteins is found specifically at centromeric heterochromatin and is required for histone methylation, Swi6 binding and gene silencing. However, this requirement has a remarkable specificity and only applies to heterochromatin at centromeric sequences, and not mating-type loci. The small RNAs correctly target to centromeric heterochromatin and, in this complex, the Argonaute protein uses the small RNA as an 'address tag' in a search for genomic regions with the same sequence where it delivers the Chp1 protein.

The finding that small RNAs can target a complex to the genomic region from which they were transcribed could throw light on the mystery of other protein complexes that bind to specific genomic sequences *in vivo*, but whose members apparently do not bind specifically to DNA.

3 Verdel, A. *et al.* (2004) RNAi - mediated targeting of heterochromatin by the RITS complex. *Science* 303, 672–676

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Immunology

The enormous effectivity of DCs in lymph nodes

Lymphocytes interact with antigen presenting cells (APC), the most effective of which are dendritic cells (DCs). Although much work on such cell-cell interactions has been performed in *in vitro* systems, it has only recently become possible to study the cells in their natural environment directly.

The group of Michael Cahalan is leading this type of analysis and a recent publication [4] focusses on driving local DCs into draining lymph nodes and imaging their interactions with T cells. The research followed up two questions: (a) how effective is the migration and motility of endogenous DCs; and (b) how effective is the interaction with T cells. Endogenous DCs showed a tremendous amount of motility and increased the area reached by processions from the DC body by a factor of 3 compared with a round spherical shape.

The authors showed that interaction with T cells was a stochastic process; no directional migration of T cells to DCs was detectable. Nevertheless, DCs were extremely efficient at contacting large numbers of T cells and use rapid touching mechanisms to scan up to 5000 T cells per hour. This number gives an idea how a small number of DCs is able to scan practically all T cells in a lymph node: 200 DCs would only need 1 h to scan the 10^6 T cells that are present in a normal mouse lymph node – a very efficient process indeed!

Future work needs to address how many DCs loaded with a given antigenic peptide–MHC complex are present within a lymph node following natural infection. We also expect further work from the Cahalan lab, as in this study DCs were not loaded with specific antigen.

4 Miller, M.J. *et al.* (2004) T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. *Proc. Natl. Acad. Sci. U. S. A.* 101, 998–1003

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