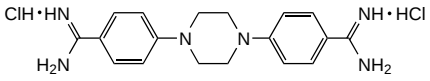
chloroquine, the parasites that cause malaria (which belong to the genus *Plasmodium*) have developed resistance to this drug. Recent research has focused on a series of novel bisbenzamidines in which the aromatic moieties are linked via a piperazine moiety to afford a conformationally restricted structure that differs from previously described analogues [3]. A small library of 11 analogues was prepared in solution as singletons. The antiplasmodial activities of the synthesized compounds were determined from the extent to which they inhibited the incorporation of [^{3}H]-hypoxanthine in nucleic acid synthesis (via the parasite purine salvage pathway). Each compound from the library was evaluated for activity against two *P. falciparum* strains (the chief



(ii)

cause of human malaria): a cloned chloroquine-susceptible strain from Haiti (Haiti 135) and a cloned chloroquine-resistant strain from Indochina (Indochina I). Results were reported as the concentrations of the test compounds required to inhibit the incorporation of [^{3}H]-hypoxanthine by 50% (IC_{50}). One of the most potent compounds identified was **ii**, which had an IC_{50} of 3 nM against the Haiti 135 strain and an IC_{50} of 4 nM against the Indochina I strain. Aromatic diamines are widely prescribed for the treatment of fungal and protozoal infections, despite their side effects. However, diamidines have not been evaluated extensively as potential antimalarials. Compound **ii** is 50-fold more active than chloroquine itself against the chloroquine-resistant Indochina I strain of *P. falciparum*. Thus, the 4,4′-(piperazine-1,4-diyl)bisbenzamidines are a promising novel class of compounds with potential antimalarial activity.

3 Mayence, A. *et al.* (2004) Parallel solution-phase synthesis of conformationally restricted congeners of pentamidine and evaluation of their antiplasmodial activities. *J. Med. Chem.* 47, 2700–2705

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Biology

Cancer Biology

Cell invasion

Several lines of evidence are growing toward the involvement of tumor–stroma interaction in cancer progression. However, pathways that lead to tumor growth, invasion and metastasis remain unfamiliar. In an article published recently in *Cancer Research* Sato *et al.* [1] used gene-expression profiling to identify the genes that are overexpressed or underexpressed during tumor growth. In a coculture of fibroblasts and pancreatic cancer cells, the tumor cells became more invasive, which is indicative of a change in gene-expression pattern.

Out of the 18,000 transcripts analyzed, <0.8 % showed either up- or downregulation in each cell type. Many genes that were pulled out via gene profiling remain to be identified, and their role in tumor–stroma interaction characterized. Nonetheless, a number of the upregulated genes identified in the current study were already known to be involved in cell migration and tumor invasion. Similarly, the downregulated genes are known to impede cancer progression.

Sato *et al.* confirmed the expression level of a subset of genes via RT–PCR. The cyclooxygenase 2 (COX-2) gene particularly attracted the attention of the authors: COX-2 is upregulated in pancreatic tumor cells and fibroblasts. It is known to be implicated in tumor metastasis and angiogenesis, and many known COX-2 inhibitors can impede cell proliferation. Thus, they addressed the effect of COX-2 inhibitor on tumor–stroma interaction and showed that the presence of specific COX-2 inhibitors in a coculture of pancreatic cancer cells and fibroblasts limits cell invasion in a concentration-dependant manner.

1 Sato, N. *et al.* (2004) Gene expression profiling of tumor-stromal interactions between pancreatic cancer cells and stromal fibroblasts. *Cancer Res.* 64, 6950–6956

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Making the transition at a Snail's pace

Epithelial-mesenchymal transition (EMT) is an important process during development that has also been implicated in metastasis. A key event is repression of E-cadherin by transcriptional regulators such as Snail. Although Snail mRNA can be present in cancer cells, the protein is often undetectable. This observation prompted researchers to investigate pathways modulating Snail protein stability [2]. Treatment of cell lines with MG132 (a proteosome inhibitor) and lithium (a GSK-3β inhibitor) resulted in synergistic elevation of Snail. This suggests that the amount of Snail protein is regulated by GSK-3β phosphorylation and proteosomal degradation.

Two GSK-3β phosphorylation motifs were identified in Snail. Site 1 overlapped a degradation site recognised by β-Trcp, whereas Site 2 appeared to enhance nuclear export. A dual-control model for regulating Snail protein stability was therefore elaborated and tested in a series of *in vitro* studies. In essence, GSK-3β binds nuclear Snail and phosphorylates Site 2, resulting in export to the cytoplasm. Subsequent modification of Site 1 by GSK-3β allows recruitment of β-Trcp and proteosomal degradation.

Numerous oncogenic signals inhibit GSK-3β. In this study, EGF abrogated the GSK-3β mediated effects on Snail. Many cancers express high levels of the EGF receptor. It is therefore possible that metastasis requires suppression of GSK-3β, resulting in stabilization and nuclear accumulation of

1359-6446/04/$ – see front matter PII: S1359-6446(04)03192-7

Snail, with an associated downregulation of E-cadherin. This has yet to be formally tested; however, the results presented in this report give a tantalizing glimpse of the mechanisms driving cancer cell invasion.

2 Zhou, B.P. *et al.* (2004) Dual regulation of Snail by GSK-3β-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat. Cell Biol.* 6, 931–940

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Structural Biology

The structure of a human histone deacetylase suggests possibilities for novel anti-tumour compounds

The level of acetylation of histones affects the electrostatic interactions between these proteins and DNA. Therefore histone deacetylases (HDACs) and acetylases are involved in the epigenetic regulation of gene expression. Alterations in gene expression can lead to cancer and inhibitors of HDACs have been shown to have anti-tumour activity. To date there have been no structures of the eukaryotic HDACs that these inhibitors target, so it is not clear how they work. Vannini *et al.* have now solved the structure of human HDAC8 bound to a hydroxamic acid inhibitor.

There are three classes of HDACs and classes I and II are zinc-dependent. The HDAC inhibitors are largely non-specific and target most of the class I and II enzymes, but it is not known whether the potency of these compounds is due to inhibition of a large number of HDACs or whether it is just a few key enzymes. Therefore the authors used RNA interference to target HDAC8 in several human tumour cell lines. They found that cell proliferation was inhibited in all the cell lines tested, suggesting that specifically inhibiting HDAC8 has anti-tumour activity and that compounds that only target HDAC8 would be effective.

The authors tested the inhibition of HDAC8 by a range of compounds and found one in particular that targeted it. They then solved the crystal structure of human HDAC8 bound to this compound. The structure was similar to a previously determined prokaryotic deacetylase, but it differed significantly at the loop regions and the active site was much more accessible. The inhibitor coordinates the active site zinc and also contacts several of the key residues required for catalysis. The structure also suggests ways in which the binding affinity of the compound can be increased. It will be interesting to see the structures of other HDAC-inhibitor complexes in the future.

4 Vannini, A. *et al.* (2004) Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proc. Nat. Acad. Sci. U. S. A.* 101, 15064–15069

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Molecular Biology

Replicating but not relocating DNA

Before each cell division the six billion base pairs that constitute the diploid human genome must be fully and accurately replicated (at S-phase). This mammoth task is achieved by the orchestrated performance of hundreds of replication foci. However, despite extensive studies into the organization of these foci during S-phase, the question of whether DNA relocates in the nucleus, for the purpose of replication, remained unresolved. So, does DNA move to immobile replication machinery for synthesis, or does the replication machinery motor along an immobile DNA track? Alternatively, could the DNA and replication machinery both move? Sadoni *et al.* have now reported the answer in the *Journal of Cell Science* [3].

Using a fluorescent (green) PCNA fusion protein (a replication factor) together with fluorescent nucleotides (red) the group was able to simultaneously visualize both the replication machinery and the newly forming DNA in living cells. HeLa cells were microinjected with an expression plasmid for the fusion protein and with limiting amounts of fluorescent nucleotides, such that only small, localized regions of the genome were labeled. By measuring the distance between these DNA foci and between DNA foci and the nucleolar periphery (used as a relatively immobile nuclear landmark) the group observed that, both during replication (when red and green signals colocalized) and after (when red and green signals were separate, later in S-phase), the positional changes of DNA foci was minimal (not exceeding 0.5 microns in 95% of cases). Thus, DNA does not relocate to replicate.

The group also found that timing of DNA replication is maintained through cell division. This was shown by the fact that DNA foci synchronously replicated (labeled) in the mother cell were then synchronously colocalized with PCNA in the daughter cells. That timing is maintained through cell division fits well with findings that chromosome positioning is also maintained through cell division – as studies indicate a close relationship between chromosomal locus positioning and replication timing. It will be interesting now to determine the factors controlling locus positioning in the nucleus and to assess whether position determines replication timing or vice versa.

3 Sadoni, N. *et al.* (2004) Stable chromosomal units determine the spatial and temporal organization of DNA replication. *J. Cell Sci.* 117, 5353–5365

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Sweet flexibility

Potassium channels let K^+ ions passing through the plasma membrane and are essential to maintain the membrane potential of living cells. How K^+ channels are able to discriminate very efficiently against other cations and still maintain a fast rate of permeation was, until recently, attributed to the strict geometry and rigidity of their pore. Crystallographic structures of K^+ channels showed K^+ ions fitting snugly into the pore, coordinated by the backbone carbonyl oxygens from the

signature sequence of K^+ channels. But this result did not explain how the channel could maintain such a precise geometry in spite of thermal fluctuations and whether a rigid channel could enable fast conduction.

Unsatisfied by the classic explanation, Noskov *et al.* [5] pushed the study of permeation to another level. Realizing that the difference in size between K^+ and Na^+ ions is much less than the structural changes within the diameter of the selectivity filter – a deviation of 0.15 Å per subunit within the selectivity filter would be sufficient to abolish the preference for K^+ – they addressed the issue of protein flexibility and its influence on ion selectivity directly.

The authors showed, via a series of careful computations, that selectivity is partly controlled locally by the intrinsic carbonyl–carbonyl repulsion in the selectivity filter, and that there is no need for rigidly maintaining a precise pore geometry. This result demonstrates that accounting for the microscopic physical properties is crucial for understanding biological mechanisms in detail.

5 Noskov S.Y. *et al.* (2004) Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. *Nature* 431, 830–834

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Targets and Mechanisms

Bisthiazolium prodrugs with strong oral antimalarial activity

With widespread drug resistance to common antimalarial drugs, much current research is focussed on new compounds unrelated in structure to existing drugs and that act against novel drug targets. Quaternary ammonium compounds that resemble acetylcholine are active against parasite phospholipid biosynthesis. Given that the parasite has to synthesize large quantities of lipid membrane in the course of its 48-hour blood cycle, this represents a potentially excellent target. Unfortunately however, quaternary ammonium compounds are poorly suited as drugs owing to their permanent positive charge and hence low oral bioavailability. Now Vial and co-workers [6] have prepared prodrug precursors of two thiazolium compounds that are rapidly metabolised by human blood plasma enzymes to produce highly active compounds.

Acyclic thioester precursors of the thiazolium target molecules were synthesized and shown to be cyclized to the desired products by esterase enzymes. The metabolites exert powerful antimalarial activity *in vitro*. This ranges from 15 times greater than chloroquine in a chloroquine-sensitive parasite strain, to 200 times in a chloroquine-resistant strain. The prodrugs are similarly active, but an analogue lacking the thioester group that cannot be metabolized to a thiazolium product is essentially inactive. These compounds were found to accumulate in parasites, apparently through interaction with haem. Impressively, they show potent *in vivo* oral antimalarial activity.

6 Vial, H.V. *et al.* (2004) Prodrugs of bisthiazolium salts are orally potent antimalarials. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15458–15463

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Business

Collaborations

Dynavax establishes collaboration with Riken Institute for development of cedar allergy therapeutics for Japanese market

Dynavax Technologies Corporation (http://www.dynavax.com), a biopharmaceutical company developing treatments for allergy, infectious disease and chronic inflammatory disease, has announced the establishment of a collaboration with the Riken Institute for the development of novel cedar tree allergy therapeutics utilizing the company's proprietary immunostimulatory sequence-(ISS-) based therapeutics platform. Cedar tree allergy is a serious public health challenge in Japan, which afflicts >15M sufferers, representing approximately 12% of the country's total population, and is increasingly prevalent.

The principal investigator from the Riken Institute in this collaboration will be Masahiro Sakaguchi, Japan's leading researcher in cedar tree allergy. 'We are honored to collaborate with Dr Sakaguchi and the prestigious Riken Institute with the goal of developing a new approach to treating cedar allergy,' said Dino Dina, President and Chief Executive Officer of Dynavax Technologies. 'Based on data we have generated in multiple clinical trials for ragweed allergy, and the broad application of our proprietary ISS-based technology to a wide range of allergic disorders, we are optimistic that our efforts will lead to development of a safe and effective cedar allergy treatment that provides long-lasting relief from this serious health problem. Considering the pervasiveness of this disorder in Japan, and the need for new interventions, we believe that this collaboration has significant therapeutic as well as commercial potential for our company.'

Under the terms of the two-year collaboration, Dynavax will apply its expertise in the discovery and development of immunostimulatory sequence (ISS)-based allergy therapeutics and develop a cedar antigen-ISS conjugate product. Dr. Sakaguchi, who has performed early animal testing of these therapies with promising results, will further test these therapeutic candidates in his advanced, proprietary animal models of

1359-6446/04/$ – see front matter PII: S1359-6446(04)03177-0