the ester oxygen replaced by a methylene group and are thus not susceptible to nucleophilic attack by the active site cysteine. Some of these compounds (such as 14, 17, and 18; see Scheme 3 of Wu et al. [1]) are competitive inhibitors with $K_i$ values in the lower micromolar range and are thus among the most potent noncovalent inhibitors of the SARS-CoV M$^\text{pro}$ described to date. The discovery of compounds binding noncovalently to the M$^\text{pro}$ may, in the end, constitute a more important milestone on the way to clinically useful inhibitors of the coronavirus main proteinase than identification of the acylating agents.

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Selected Reading


Pharmacogenetics:
Yeast Lead the Way

In this issue of Chemistry & Biology, Perlstein et al. [1] use genetically diverse strains of yeast to study the genetic basis of differences in cellular responses to small molecules. Their results suggest that drug responses are regulated by a limited number of loci, and that this system can identify clusters of functionally similar molecules.

When given a standard dose of commonly prescribed drugs, a significant fraction of patients will either receive...
no therapeutic benefit or will experience an undesired side effect. Pharmacogenetics is the study of the genetic basis of variation in responses to drugs, which is thought to account for a large fraction of differences in drug responses among human populations [2]. Perlstein et al. explore the issue of how genetic differences affect drug response in outbred yeast strains, and they do so with a level of experimental rigor impossible in other systems [1].

To initiate this study, two yeast strains were used: a standard laboratory strain and a strain isolated from an AIDS patient [3]. The entire genome of both strains has been sequenced. The two strains exhibit $10^{-9}$ changes per synonymous site, a level of genetic divergence comparable to that between humans and chimps [4]. The authors first identified 23 small molecule perturbagens (SMPs) that were differentially toxic to three different diploid strains of yeast: the homoyzogous laboratory strain, the homozygous clinical isolate, and a heterozygote between the two strains. The SMPs, split roughly equally between natural products and synthetic chemicals, include seven FDA-approved drugs. Differential toxicity among these strains ensured that there would be interesting pharmacogenetic differences between the progeny of the heterozygous diploid.

The authors sporulated the heterozygous diploid to generate a genetically diverse population of 2246 haploid progeny, each of whose genomes contained a unique combination of alleles inherited from the clinical strain and the laboratory strain. A sensitivity profile to the 23 SMPs was determined for each haploid strain by measuring the degree to which each compound inhibited growth.

To begin to address the issue of pharmacogenetic complexity, the authors estimated the number of genetic loci that control sensitivity to each of the 23 SMPs. The distribution of sensitivities to a certain drug across all progeny is expected to reflect the number of loci that influence sensitivity to that drug. For example, if half of the segregants are resistant to a certain SMP and half are sensitive, it is likely that a single locus controls sensitivity, whereas if three quarters are resistant and one quarter are sensitive, it is more likely that two loci control sensitivity. Assuming that the fraction of resistant segregants approximates $(1/2)^n$, the authors determined that sensitivity to all SMPs was controlled by no more than four loci, and only one SMP, flunarizine, was controlled by only a single locus. Interestingly, there was a kinetic component to resistance. For example, although sensitivity to flunarizine appeared to be controlled by only one locus when growth inhibition was measured at 48 hr, it appeared to be controlled by two loci 24 hr later. This kinetic component was seen with several SMPs, emphasizing the pharmacogenetic complexity in the population.

The fact that the numbers of genetic determinants appeared to vary from one to four across the 23 SMPs was consistent with the idea that they reflected a large number of genetic differences that affected sensitivity to a small number of drugs rather than the converse. The pattern of sensitivity to different SMPs among the segregants, analyzed by two-dimensional hierarchical clustering demonstrated that the genetic determinants controlling sensitivity to most SMPs segregated independently of each other.

Although the majority of SMPs elicited unrelated patterns of sensitivity, principal component analysis (PCA) identified clusters of SMPs that elicited similar patterns. PCA is a method of dimensionality reduction that places the SMP in three dimensional “chemical space” according to the pattern of segregant sensitivity, and can therefore be used to measure the relatedness between different SMPs and group them into clusters. Prior knowledge of the biological effects of the SMPs in three of these clusters confirmed that this mathematical clustering was biologically meaningful. One cluster consisted of five molecules that, although structurally unrelated, all modulate calcium ion homeostasis. A second cluster consisted of four molecules known or postulated to modulate respiratory metabolism. A third cluster consisted of two molecules that, although structurally different, both inhibit calmodulin, a master regulator of calcium-mediated signaling. These results suggest that principle component analysis of the patterns of sensitivity elicited in genetically diverse populations of yeast could prove a powerful tool for identifying compounds whose biological effects are similar to those of known compounds but whose structure, and perhaps mechanism of action, are unrelated.

Analysis of the patterns of sensitivity elicited by these SMPs proved valuable not only for identifying molecules with similar biological activities, but also for identifying interactions between molecules and the biological processes on which they impinge. For example, principle component analysis demonstrated that two SMPs that modulate calcium homeostasis reside at the opposite corners of PCA-defined chemical space from three SMPs that modulate respiration. This relationship is manifested in an underrepresentation of segregants that are resistant to both the modulators of calcium homeostasis and the modulators of respiration, and it suggests an interaction between the two biological processes. This hypothesis was further strengthened by treating segregants that were resistant to SMPs from both classes with a combination of one SMP from each class; although these strains were not sensitive to either class of SMPs, the combination of one from each class completely inhibited growth. This result suggests that patterns of sensitivity elicited in genetically diverse populations of yeast could prove a powerful tool for identifying toxic interactions between drugs.

Finally, the authors demonstrated that their approach was, in fact, identifying genetic loci that regulate drug responses by mapping some of the loci responsible for modulating drug responses. To accomplish this, they turned to a cross between the laboratory strain and densely genotyped agricultural isolate [5] whose genome is comparably divergent from the laboratory strain as is the clinical isolate.

The observation that most responses to drugs were controlled by four or fewer genes combined with the fact that the authors were able to map loci that controlled these responses is encouraging for the prospects of progress in pharmacogenetic studies in humans. The CEPH cell lines (http://www.cephb.fr/) are a promising resource for initiating a comparable study in human cells. These densely genotyped cell lines were collected...
from large Mormon families whose lineages and histories of disease are well documented and have been used successfully for mapping several quantitative traits such as gene transcript levels [6]. Furthermore, the single nucleotide polymorphisms represented in this collection, including those in genes that participate in drug metabolism [7], are commonly found in the rest of population. Systematic identification of the alleles that control drug responses in humans will be an important step toward individualized drug therapy.

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