7.4.2 Whole Organism Screening

Often referred to as "phenotypic screening," as well, the screening of natural products in whole organisms was historically the original mode, and one that still has a great deal of potential. One of the most robust examples of this approach is screening for antimicrobial activity by inhibiting the growth of selected strains of bacteria on seeded agar plates. A positive response in such an assay is observed as a zone of inhibition of growth around a test sample deposited on the Petri plate. In most cases, the diameter of the zone of inhibition is directly related to the concentration of the antibacterial agent in the test sample, so that some degree of quantification of the response is also possible in this simple test. Such assays provide no indication of the mode of action of the active principle, but are generally quite effective tools for following fractionation processes that lead to pure compounds. A crucial extension of this in vitro whole organism assay is another phenotypic test in which the isolated material is administered to rodents that have been challenged with a lethal infection of the target bacterium. The positive end point of the in vivo rodent test is survival beyond that of the control animals. Positive results in the in vivo model of infection provides the critical information that the compound has sufficient drug-like properties to penetrate the normal xenobiotic defenses of the host animal and reach the target population of infecting bacteria.

There are many such whole organism models that have been used for drug screening. Among the simplest of these are those related to infectious diseases, including the aforementioned antibacterial system with various classes of pathogenic agents, as well as those designed for antifungal, antiviral, and antiparasitic agents. In the quest to find new effective agents against cancer, animal models of disease remain a mainstay of the process. Similar models are the norm for advancing the development of drugs in terms of understanding the efficacy, tolerability, metabolism, and long-term effects. These systems are rarely used for high-throughput screening of crude natural products because they require substantial resources to maintain. Therefore, most live animal models are used to verify the efficacy of compounds that have been isolated with the aid of a simpler in vitro assay.

As will be discussed in the following section, screening against isolated target biomolecules, such as enzymes or receptors is now favored for high-throughput screening operations. In the case of screening mixtures of natural products, however, the whole organism approach offers tremendous advantages. The discovery of a novel secondary metabolite that confers a positive response in a whole organism screen provides the opportunity to discover a new target, and potentially a new mechanism of action. In current parlance, these studies are often referred to as "chemical biology" or "chemical genetics" (see also Chapter 4). Specifically, in forward chemical genetics, a small molecule, in our case a natural product, is employed to probe for their cellular targets. Typical experiments include the creation of affinity binding reagents or affinity matrices that include the small molecule of interest and these systems are used to fish out target macromolecules from cellular components. Molecular targets for rapamycin and geldanamycin were found by such methods. Once the target macromolecules are verified, additional mechanistic studies are developed to understand the relationship between the binding partners and the disease process.

7.4.3 Target-Based Screening

Molecular biology has provided the tools to engineer and produce macromolecular targets of drug action. If it is believed that the inhibition of a particular cell-signaling process will mediate the development of disease, then the isolated enzyme or receptor that is responsible for the signaling can be used as a target for screening. Alternatively, a selective whole cell screen can be employed which is designed to respond by providing some measurable signal as a result of the interaction with a particular target. Owing to developments in automation for such assay systems, hundreds of thousands of compounds can be conveniently tested for activity in a short period of time. Natural product mixtures may also be tested in these highly automated systems. With mixtures, particularly