



# Cell-Based High-Throughput Screens for Drug Discovery

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**By Dr Alcide Barberis, Chief Scientific Officer of ESBATech AG**

**Dr Alcide Barberis is the Scientific Founder of ESBATech, a drug discovery company specialising in cell-based functional screening assays and high-throughput compound screens. Dr Barberis received his PhD in Molecular Biology and Biochemistry from the University of Zurich in 1988. He later moved to Harvard University as a postdoctoral fellow, where he began working with yeast as a model system to study the fundamental mechanisms of gene regulation. After a period at the Research Institute San Raffaele in Milan, he became group leader at the Institute of Molecular Biology at the University of Zurich. In 2002 he left academic research to become full-time Chief Scientific Officer of ESBATech.**

The rapid progress in elucidating the mechanisms underlying human diseases, along with the enormous amount of data generated by the Human Genome Project, are significantly increasing the number of proteins that can be targeted for potential drug treatment. Parallel to these developments, the number and diversity of compounds that can be tested for activity against these targets are also rapidly expanding. The combination of these trends in biology and chemistry has spurred the development of increasingly rapid, selective and reliable high-throughput screening assays to be applied in the early phase of drug discovery. Typically, such assays allow screening of large numbers of compounds in an automated fashion to identify those that show an activity, either as agonists or as antagonists, on a defined biological target. Most of the assay formats developed to date can be grouped into cell-free (biochemical) or cell-based (cellular) assays. This article describes the applicability of cell-based assays in the early phase of drug discovery, with a particular focus on the use of yeast cells as an attractive option for high-throughput screens.

Prior to the recombinant DNA revolution and the advent of biotechnology, drug discovery was largely dependent on mass screenings using whole organisms or cells to evaluate biologically active substances without knowledge of their potential molecular targets. With the development of new techniques to identify genes and proteins and increased insight into the molecular mechanisms underlying disease, a target-based approach to drug discovery using mechanism-based screening assays has replaced the old non-specific assays. In order to enable the rapid analysis of large numbers of compounds for their effects on the function of specific targets, so-called high-throughput screening assays have been developed.

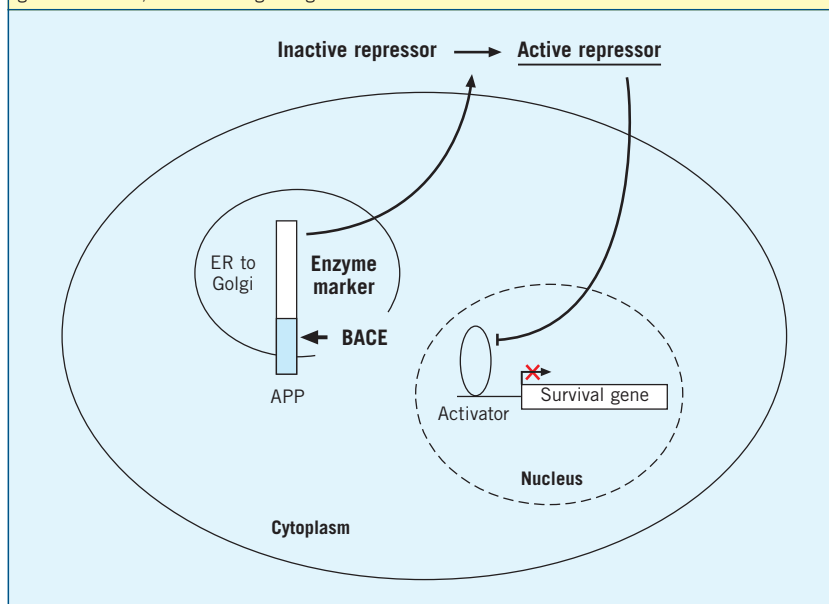
High-throughput screenings (HTS) are generally performed robotically. Thus, applicable protocols must require minimal manipulation, possibly in arrayed small volumes, such as those provided by 96-well microtitre plates, which represent a standard format for automated assays. Many types of cell-free assays that were originally developed to measure the biochemical activity of purified proteins, mostly enzymes,

could be readily converted to HTS by applying detection systems that do not require separation of the reaction product from substrate. For example, fluorescence-based techniques allow detection of enzymatic activities as well as of molecular interactions without the requirement of separation, fractionation or purification procedures (1). These automated assays allow rapid screens of large compound libraries to identify so-called 'hits', namely compounds that show the desired effect on the biochemical activity of the specific target in the isolated *in vitro* system. Hits are then subjected to chemical modifications and further screening through the HTS system to select more specific and potent derivatives called 'lead' compounds. In the classical drug discovery process, lead compounds are subsequently tested in various *in vivo* assays using cellular and animal models in order to select those that may become drug candidates for clinical trials (2).

In the last few years, cell-based assays using engineered cells and microorganisms have become an increasingly attractive alternative to *in vitro* biochemical assays for HTS in the early phase of the drug discovery process. The requirements for such *in*

**Figure 1: Yeast Selection System for Identifying *In Vivo*  $\beta$ -Secretase (BACE) Inhibitors**

BACE substrate, the membrane-anchored APP, is expressed in yeast as a fusion protein with an enzyme marker. Both BACE and APP fusion proteins are localised in ER and Golgi membranes. Cleavage of APP by BACE causes release and subsequent secretion of the enzyme marker. Secreted enzyme can catalyse production of a transcriptional repressor, which, upon uptake by the cell, blocks activation of a survival gene resulting in growth inhibition. A compound that can inhibit BACE activity in these intracellular compartments prevents secretion of the enzyme and therefore formation of the active repressor. In this case, activation of expression of the survival gene can occur, thus enabling cell growth.



*in vivo* assays are the ability to examine a specific cellular process triggered by a defined target and a means to readily measure its output in a HTS system. The availability of an increasing number of biotechnological tools to genetically modify cells and microorganisms has allowed the development of simple read-out assays for cellular processes that can be readily applied to automated systems in HTS (1-4). Cell-based assays have notable advantages over *in vitro* assays. Firstly, these *in vivo* assays do not require purification of the target protein and therefore eliminate investment of resources to gain the necessary knowledge for obtaining a biochemical active target – this advantage has become particularly important with the increasing number of proteins that can be targeted for potential drug treatment as it would indeed be difficult to set up specific biochemical assays for hundreds of new proteins for which the natural substrates remain largely unknown. Secondly, the conformation and the activity of the target protein, as well as the read-out to monitor the effect of compounds, are examined in a cellular context that most likely represents the natural physiological state more closely than *in vitro* assays. Thirdly, cell-based assays can immediately select against compounds that are generally cytotoxic, or that cannot permeate cellular membranes to reach intracellular targets. Thus, hit and lead compounds that are identified through cell-based assays have passed important validation steps. The availability of this information provides a head start compared to many *in vitro* assays and can save valuable time and costs in the development of the drug.

Cellular screens should ideally be performed with cells of human origin, which evidently provide the most physiologically relevant model system. However, the effects of redundant processes on the measured output can be difficult to control and

to distinguish from the effects that are expected to be specific for the defined target; and genetic manipulation of mammalian cells is generally problematic and time-consuming. Moreover, human cells are expensive to culture and sometimes difficult to propagate in automated systems used for HTS.

Microorganisms such as yeast present a convenient alternative for measuring the activity of defined human proteins in a heterologous, yet cellular (eukaryotic) environment. In yeast cells, the function of human proteins can often be constituted and aspects of some human physiological processes can be produced because of the high degree of conservation of basic molecular and cellular mechanisms between yeast and human cells (4-7). The fact that many human proteins function in yeast indicates that the required conformation, stability, protein-protein interaction and so on, are taking place in this eukaryotic organism. This is a fundamental notion for the application of yeast cells in HTS with human target proteins. On the other hand,

despite the high degree of similarity of basic cellular processes between yeast and human cells, yeast shows differences that might impair attempts to reproduce the activity of some human targets and to test some potential drug candidates. Thus, yeast may not be used for screening against every human target. However, having assessed this, the employment of yeast in cell-based assays for HTS admittedly has several advantages.

One major advantage over mammalian cells is the versatile genetic malleability of this organism, which has made it the model of choice for pioneering research in molecular and cell biology over the last three decades (8). This characteristic of yeast, as well as long scientific experience in yeast genetics and molecular biology, has allowed the development of a multitude of genetic tools and cellular selection systems that can now be readily converted to HTS formats. These molecular and cellular tools include advanced plasmid systems, homologous recombination techniques and the selection of readily scored markers, such as nutritional selection based on the piloted activity of, for example URA3, HIS3, LEU2 and other genes, drug resistance (for example *kan<sup>r</sup>*, *nat<sup>r</sup>*), drug or compound sensitivity (for example *cyh<sup>r</sup>*, GAL1, URA3) and colorimetric selection (for example LacZ, GFP, Luciferase). One additional advantage of this eukaryotic organism for cell-based HTS is the low cost of growing and manipulating yeast. Moreover, in contrast to mammalian cells, where redundant processes often prohibit the measurement of a specific effect on a given target, a heterologous yeast system permits a clean read-out in a null-background environment for the expression of the human protein. Finally, although yeasts are surrounded by a cell wall and their plasma membrane composition differs to some extent from that of their mammalian counterparts, any compound that

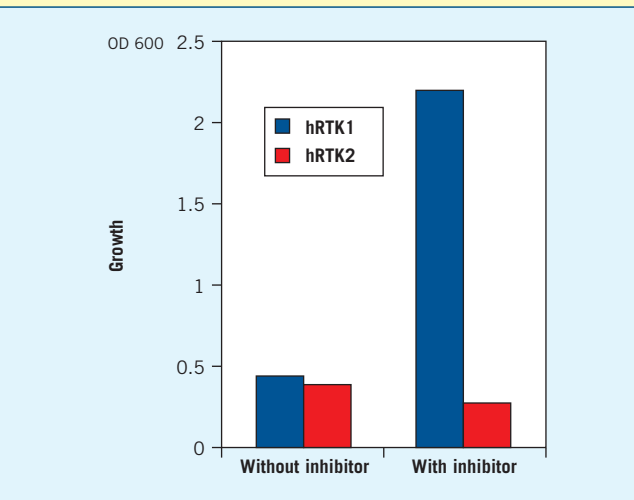
can penetrate a yeast cell should be able to enter a human cell. It has become apparent that the cell wall itself does not limit permeability of small molecules, and that compound efflux rather than membrane penetration is the mechanism that may lower the intracellular concentration of compounds. In yeast, this process can be readily controlled and its effect dramatically reduced by deleting the major ABC transporters (9).

Thus, the possibility of reproducing human protein function in the cellular environment of a heterologous eukaryotic organism and the genetic versatility of yeast have prompted researchers to set up target-specific HTS assays with engineered yeast cells. For example, active mammalian ion channels have been reconstituted in yeast to yield a readily assayed growth response for HTS, either by complementing growth phenotypes of yeast mutants defective in ion transport, or by causing growth defects when expressed in engineered yeast strains (10, 11). In a different approach based on the so-called reverse two-hybrid system applied to an HTS format, interactions between ion channel subunits that are essential for their function have been reconstituted, and compounds that can disrupt such interactions have been identified by yeast growth selection (7, 12). Such inhibitors of specific protein-protein interactions have subsequently been shown to block the whole channel activity in functional assays (7, 12). A further example is provided by screening assays with human G-protein-coupled receptors (GPCRs). GPCRs have indeed been functionally expressed in yeast, in which they can substitute for the structurally similar Ste2p, the yeast mating pheromone receptor. Thus, ligand binding to the human GPCR can activate the yeast pheromone-signalling pathway and trigger induction of mating-specific genes as well as activation of selectable reporter genes bearing mating-specific promoter elements. Such engineered yeast cells have been used in HTS to select specific agonists as well as antagonists of GPCR signalling (13, 14).

Researchers in our laboratories have established a cellular growth selection system for functional expression of the human  $\beta$ -secretase BACE in yeast, in which processing of the human transmembrane amyloid precursor protein (APP) by this membrane-bound protease in the ER-Golgi lumen is readily detected (15, 16). These yeast cells have been engineered so as to grow under selective conditions only upon reduced or inhibited BACE activity (see Figure 1). Thus, these cells can be used in HTS assays to select for compounds that inhibit this transmembrane secretase acting on its natural substrate in a membrane-bound protein configuration, a condition that would be very difficult to achieve in a cell-free assay. A conceptually similar approach has been developed to identify specific inhibitors of human receptor tyrosine kinases (RTKs). In this system, yeast cells expressing human RTKs are enabled to grow only upon inhibition of their kinase activity. Administration of a specific tyrosine kinase inhibitor to the yeast cells was indeed shown to block the kinase activity of a defined human RTK target and to restore growth under selective conditions (see Figure 2). Growth inhibition caused by a different RTK

## Figure 2: Selection of Specific Inhibition of RTK Activity in Yeast

Expression of human receptor tyrosine kinases hRTK1 and hRTK2 in yeast causes retardation of cell growth, as determined by OD600 light scattering measurement of both cell cultures. Addition of an hRTK1-specific kinase inhibitor to these yeast cultures specifically restores growth of hRTK1-expressing yeast cells but not growth of cells expressing hRTK2.



(hRTK2), which is known to be unaffected by the specific kinase inhibitor, was not relieved in the presence of this compound (see Figure 2). Since yeast cells do not have endogenous mammalian-type tyrosine kinases, this system offers the advantage of a null background for the expression of human RTKs and for the screening of specific inhibitors of these membrane-bound kinases.

In summary, while cell-free assays can be very rapid and allow the use of clear-cut, simple read-outs that specifically monitor the activity of the isolated target, cell-based assays provide a cellular environment that produces the natural physiological state of the target and its substrate or interacting partner more accurately. The easy genetic manipulation of yeast and the high degree of conservation of basic molecular mechanisms have made this eukaryotic microorganism a valuable system for target-specific drug screening. The decision about the best type of assay that should be used for a HTS with a particular target primarily depends on the biological and biochemical characteristics of the target under investigation. ♦

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