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Technology Review

High Throughput Assay Technologies for Ion Channel Drug Discovery

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Abstract: Ion channels represent a class of membrane spanning protein pores that mediate the flux of ions in a variety of cell types. To date, >400 ion channels have been cloned and characterized, and some of these channels have emerged as attractive drug targets. Several existing medications elicit their therapeutic effect through the modulation of ion channels, underscoring the importance of ion channels as a target class for modern drug discovery. To meet the increasing demand for high-throughput screening of ion channels, assay technologies have evolved rapidly over the past 5–10 years. In this article, the authors review the technologies that are currently used for the screening of ion channels. The technologies discussed are binding assays, ion flux assays, fluorescence-based assays, and automated patch-clamp instrumentation.

Introduction

Ion channels are membrane-spanning proteins that form pores through which inorganic ions, such as Na^+, K^+, Ca^{2+}, and Cl^-, can rapidly traverse the cell membrane down their electrochemical gradient (Table 1). It is well established that ion channels play a vital role in neuronal signal transduction, neurotransmitter release, muscle contraction, cell secretion, enzyme activation, and gene transcription. To date, upwards of 400 different human ion channel genes have been identified. Mutations that disrupt or alter channel function have been associated with many diseases ("channelopathies"), including hypertension, cardiac arrhythmia, diabetes, cystic fibrosis, and a variety of neuronal disorders.[1–5] Thus, ion channels represent an important class of molecular targets for drug development.

The role of ion channels in drug safety has also emerged as an important issue in the last several years. Since 1985, five drugs have been withdrawn from the market because they prolong the cardiac QT interval and, in several cases, produce a lethal ventricular arrhythmia known as *torsade de pointes*.[6–8] The mechanism underlying this toxic effect involves inhibition of one or more of the cardiac ion channels: (1) the hERG potassium channel (I_{kr}); (2) the KCNQ1/KCNE1 potassium channel (I_{ks}); and (3) the SCN5A sodium channel.[9,10]

Ion channels can be broadly grouped into two major classes[11,12]: ligand-gated and voltage-gated ion channels (Table 2). The primary distinguishing feature is that voltage-gated ion channels do not have endogenous ligands, but are activated by changes in the membrane potential. Ion channels can exist in multiple states such as the closed, open, and inactivated states. Voltage-gated ion channels transition (gate) between these states in response to changes in membrane potential. In contrast, ligand-gated channels transition between these states in response to the binding and unbinding of a ligand. In the open state, ions can flow through a single ion channel pore at prodigious rates of over 10^7 ions/s. Cell-based functional assays are an essential requirement for the screening of ion channels at both the primary and sec-

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ABBREVIATIONS: AAS, atomic absorbance spectrometry; CCD, charge-coupled device; FLIPR, fluorescent-imaging plate reader; FRET, fluorescence resonance energy transfer; hERG, human *ether-go-go*-related gene; HTS, high throughput screening; IC_{50}, 50% inhibitory concentration; SPA, scintillation proximity assay.

Table 1. Ion Concentrations Inside and Outside of a Cell

	Concentration	
Ion	*Extracellular*	*Intracellular*
Na^+	145 m*M*	12 m*M*
K^+	4.5 m*M*	140 m*M*
Ca^{2+}	1.8 m*M*	0.1–0.2 μM (cytosol, resting cell) 100 μM (cytosol, stimulated cell)
Mg^{2+}	1.5 m*M*	0.8 m*M*
Cl^-	116 m*M*	4 m*M*
pH	7.4	7.1

ondary levels. Traditional methods developed for HTS of ion channels, such as binding, ion flux, and fluorescent probes, measure ion channel activity indirectly. Patch-clamp electrophysiology is regarded as the "gold standard" for measuring ion channel activity and pharmacology. Patch-clamp allows for the direct, real-time measurement of ion channel activity, but in its traditional format is low-throughput and requires a high degree of operator skill. Hence, drug screening assays for ion channels, in comparison to those for enzyme and receptor targets, have compromised data quality for throughput. Recently, a number of new screening technologies have been developed and improved for ion channel assays and are poised to change this.[13–15] A summary of the currently available screening technologies is listed in Table 3.

Demands on Ion Channel Screening Assays

The current demands for ion channel screening in the drug discovery process can be grouped into three main areas: primary screening assays, secondary screening assays, and ion channel safety assessment.

Primary screening assays (HTS)

HTS has seen a tremendous advance during the last 10 years and remains the first critical step in the discovery of lead chemical structures for novel drug targets. To increase the probability of success for finding new leads from HTS, many companies have invested heavily in expanding both the diversity and quality of their compound libraries. For most mid- and large-sized companies, the library collection has grown to 400,000–1,000,000 (or more) compounds. The standard paradigms used to screen these libraries have evolved to automated 384-well or higher-density single-compound test formats. Minimal throughput of 30,000 (ideally $>$100,000) compounds/day has become the requisite. An important consideration in screening compound libraries of this size is the materials used in assay development (including dead volume during robotic screening, positive and negative controls, etc.), which can typically account for 30–60% of the total reagent and consumable requirements for completion of a single target screen.

Table 2. Classification of Ion Channels

Channel type	*Activator*	*Ion permeability*	*TM domains*[a]
Ligand-gated ion channels			
IP_3R	IP_3	Ca^{2+}	6-TM
CNG	cAMP	Na^+, K^+, Ca^{2+}	6-TM
nAChR	ACh, nicotine	Na^+, K^+, Ca^{2+}	4-TM
5-HT_3	5-HT	Na^+, K^+, Ca^{2+}	4-TM
$GABA_{A,C}$	GABA	Cl^-	4-TM
Glycine	GABA	Cl^-	4-TM
NMDA	Glutamate, NMDA	Na^+, K^+, Ca^{2+}	3-TM
AMPA	Glutamate, AMPA	Na^+, K^+, Ca^{2+}	3-TM
Kainate	Glutamate	Na^+, K^+, Ca^{2+}	3-TM
P2X, P2Z	ATP	Na^+	2-TM
Voltage-gated ion channels			
K^+ channels	membrane potential	K^+	6-TM
Na^+ channels	membrane potential	Na^+	24-TM
Ca^{2+} channels	membrane potential	Ca^{2+}	24-TM
Cl^- channels	membrane potential	Cl^-	12-TM

TM, transmembrane; IP_3, inositol trisphophate; IP_3R, IP_3 receptor; CNG, cyclic nucleotide-gated; cAMP, cyclic adenosine monophosphate; ACh, acetylcholine; nAChR, nicotinic ACh receptor; 5-HT, 5-hydroxytryptamine (serotonin); GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid.

[a]The pore-forming subunit of these channels.

Table 3. Screening Technologies for Ion Channel Targets

Assay type	*Throughput*	*Format*	*Cost per well*[a]	*Comments*
Membrane binding	Medium/high	96-well[b]	Median-high	Limited by ligand availability and structure. Not functional
		384-well (SPA)	Median	
Electrophysiology				
Voltage-clamp/patch-clamp	Very low	Single cell[b]		Classic and gold standard
IonWorks HT	Low-medium	384-well[b]	High	Low seal resistance (GΩ)
PatchXpress 7000	Low	16-well[b]	Very high	GΩ seal but low throughput
Ion flux assay				
Radioisotopes ($^{45}Ca^{2+}$, $^{22}Na^{+}$, $^{86}Rb^{+}$)	Low	96-well[b]	Median	Low signal-to-noise ratio, not for HTS
Rb flux assay (atomic absorbance)	Medium	96/384-well[b]	Low	For K^{+} channels
Fluorescence dye				
Ca^{2+} dye	High	96/384-well[b]	Low–median	Limited for Ca^{2+} permeable channels
Membrane potential				
Membrane potential kit	High	96/384-well[b]	Median–high	IC_{50} shifted to the right in certain types of channels
FRET-based	High	96/384-well[b]	Low–median	

[a]Cost per well is calculated only for the special consumable reagent (e.g., SPA beads, dye, or special plate/chip) based on the highest plate density available.
[b]Heterogeneous assay (cell wash is required).

Secondary screening assays (HTS hit confirmation and lead optimization)

The throughput requirement for these types of assays is much lower than HTS, but the demands on data quality are higher. Unlike primary screening, compound titration with eight to 10 different concentrations in duplicate is usually needed to determine the IC_{50} value for each compound tested in a secondary screen. Often one or more different types of assays are performed to confirm the activity of a compound. The required screening throughput for secondary assays is on the order of tens to hundreds of compounds per day.

Ion channel safety assessment

Cardiac ion channel safety has received a lot of attention in the past 5–7 years. Identifying compounds that have the potential to produce QT prolongation early in the development process is of industry-wide interest. Inhibition of cardiac hERG channels has been identified as the mechanism underlying the cardiac toxicity of several therapeutic agents. Considerable efforts have been devoted to develop a reliable high-throughput assay for these channels. Because of public health safety concerns, the U.S. Food and Drug Administration currently requires that the activity of all novel agents for which an investigational new drug application is filed be evaluated against the hERG channel. Therefore, hERG assays must be of the utmost quality, reproducibility, and reliability. Practically, the throughput requirement for these assays must be similar to secondary screening assays.

Current Ion Channel Screening Technologies

Radioligand binding assay

The radiolabel ligand-binding assay was developed in the 1960s. It has been extensively used for drug screening of many targets, including ion channels. Binding assays were utilized most extensively in the 1980s and 1990s before cell-based functional assays were made available for HTS. Binding assays incorporate the use of a ligand that is labeled with a radioactive tracer, such as ^{3}H or ^{125}I. Binding of the labeled ligand to a specific site on a channel protein can be displaced by an unlabeled compound if it binds to the same site on the protein. The activity of the unlabeled compound can be quantified by its ability (IC_{50}) to compete with the labeled ligand. Filtration binding assays utilize a glass fiber filter-mounted 96-well plate to separate free ligands with the ligand–channel protein complex. This assay requires a plate wash step, which limits the screening throughput. The SPA uses solid scintillant-containing beads to capture cell membranes. The labeled ligands bind to these membrane-coated beads, which enables homogeneous detection due to the transfer of energy from labeled ligands to SPA beads in proximity. This SPA binding assay can be miniaturized into 384- and 1,536-well formats with a throughput of 50,000–100,000 compounds per day at moderate cost.

Binding assays provide no information about the effect of novel agents on ion channel function. For example, an agonist cannot be distinguished from an antagonist in a binding assay. Additionally, if a compound interacts with the channel protein at a site distinct from the labeled ligand, it will not be detected in a binding assay. An example of this is the hERG ^{3}H-MK-499 binding assay. It has been reported that the potency of certain compounds in the ^{3}H-MK-499 binding assay can differ by more than 100-fold when compared to the potency of the same compounds measured in a functional voltage-clamp hERG assay. Voltage-gated ion channels do not have endogenous ligands, and hence exogenous toxins or compounds are used as the labeled ligands. The structure diversity of hits identified by binding assay-based primary screens for ion channel targets is often limited.

Fluorescent dye probes: calcium-sensing dye

Fluo-3 and Fluo-4 are the most commonly used fluorescent dyes for the measurement of changes in intracellular calcium. Cells are loaded with the dye, and an exogenous stimulus is applied to elicit the influx of calcium ions. The dyes are excited at a wavelength of 480 nm and emit a strong signal at 525 nm (Fig. 1). The fluorescence intensity of these dyes increases proportionally with the elevation of intracellular free calcium concentration. In a non-stimulated cell, the intracellular free calcium concentration ($\sim$0.1–0.2 μM) is four orders of magnitude less than the extracellular calcium concentration (2 mM). In contrast, the intracellular and extracellular concentration difference between K^{+}, Na^{+}, and Cl^{-} ions is much smaller ($<$20-fold) and does not change radically when cells expressing channels that conduct one or more of these ions are stimulated. Hence, fluorescent dyes that are sensitive to these ions are not widely utilized. In cells that express channels that conduct Ca^{2+}, influx of Ca^{2+} through open channels in the cell membrane can produce large changes in intracellular Ca^{2+} concentrations. These changes in intracellular Ca^{2+} concentration can be detected with the Fluo dyes. The FLIPR system (Molecular Devices, Sunnyvale, CA), developed in the early 1990s, utilizes a CCD-camera-based system to detect and capture the fluorescent signal emitted by the dyes. Throughput in this screening platform has been maximized with the addition of a fluorescence quenching substance to the assay buffer to suppress extracellular dye fluorescence, thereby eliminating the need for a cell wash step. Throughput of up to 80,000 data points/day in 384-well format with relatively low reagent cost can be achieved. A similar platform, the Functional Drug Screening System (FDSS; Hamamatsu, Hamamatsu City,

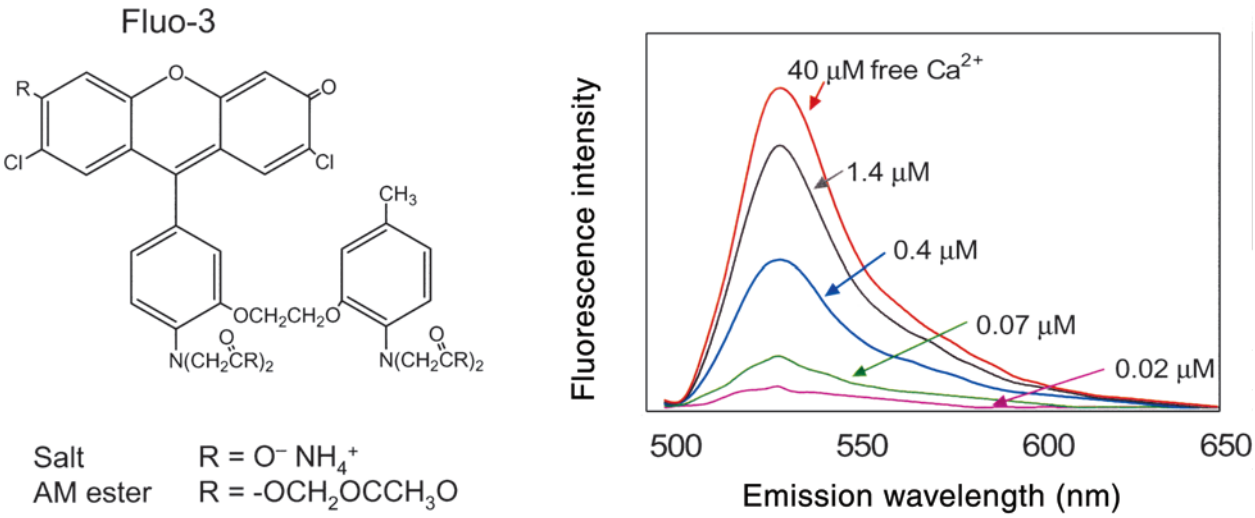

FIG. 1. Structure (**left panel**) and spectrum (**right panel**) of Fluo-3, a calcium-sensing dye. Fluo-3 is added as the membrane-permeable, non-fluorescent acetoxymethyl (AM) ester form in the loading buffer. In the cytosol, endogenous esterases hydrolyze it to form a free acid (salt form), which is not membrane-permeant and becomes fluorescent in the presence of calcium with excitation at 488 nm and emission at 525 nm.

Japan), is also available for this type of assay. Calcium-sensing dyes have been used extensively for voltage-gated channels and ligand-gated receptors that conduct Ca^{2+} ions. Since the membrane potential is not controlled in fluorescence-based assays, the potency of compounds that display "state-dependent" or "voltage-dependent" antagonism can be significantly weaker when compared to potencies obtained in the patch-clamp assay.

Fluorescent dye probes: voltage-sensing dyes

Voltage-sensing dyes are used to track changes in membrane potential. Oxonol derivative voltage-sensing dyes are negatively charged and associate with the outside layer of a hyperpolarized cell membrane. When the cell membrane is depolarized (i.e., the inner layer of the cell membrane becomes more positively charged), the dye moves into the inner layer of cell membrane. Oxonol voltage-sensing dyes were discovered in the 1960s but were not commercialized for use in large-scale screening of ion channels until the mid-1990s when plate readers became available.[16,17]

FRET-based voltage-sensing dye. FRET incorporates the use of a pair of dyes to monitor changes in membrane potential.[18,19] The FRET donor is a coumarin dye linked to a phospholipid that inserts into the outer leaflet of the cell membrane, and the FRET acceptor is an oxonol derivative. In a hyperpolarized cell, 400 nm excitation of the coumarin produces FRET and excites the oxonol derivative, also associated with the outer cell membrane, which then emits a fluorescent signal at 580 nm. When the cell membrane is depolarized, the oxonol derivative moves into the inner layer of the cell membrane, thereby creating a greater physical distance between it and the coumarin dye. FRET is disrupted because of the physical distance (>100 nm) between the two dyes. Under these conditions, the emission from coumarin (460 nm) is enhanced while the emission from the oxonol is reduced (Fig. 2). The events are quantified as a ratio of emission detected from the FRET donor and FRET acceptor. FRET-based voltage dyes can provide a relatively rapid temporal resolution (approximately seconds) in comparison to calcium-sensing dye (approximately minutes). The radiometric measurement of change in membrane potential helps to reduce assay artifacts. The Voltage Ion Probe Reader (VIPR, Aurora Discovery, San Diego, CA) was specifically designed for FRET-based assays and has a throughput of 35,000–50,000 compounds per day (384-well format). Drawbacks to this approach include (1) special and costly instrumentation is required, (2) the dyes are only capable of monitoring slow membrane potential changes and thus their use is limited to a select group of ion channels, and (3) it requires two cell wash steps, which places a limit on throughput. A multiwavelength FLIPR instrument that can collect data using radiometric dyes was recently introduced (FLIPR^{Tetra}, Molecular Devices). This instrument collects images at different wavelengths in rapid succession (<1 s) rather than simultaneously.

A proprietary membrane-potential dye kit (Molecular Devices). This kit has been used for the homogeneous measurement of changes in membrane potential with several potassium channels.[20,21] It utilizes a voltage-sensing dye mixed with proprietary fluorescent quenchers. The temporal resolution of this dye is in the range of minutes, slower than the FRET-based voltage-sensing dye combination. Throughput is maximized by the use of a quencher, which enables homogeneous assay format. The

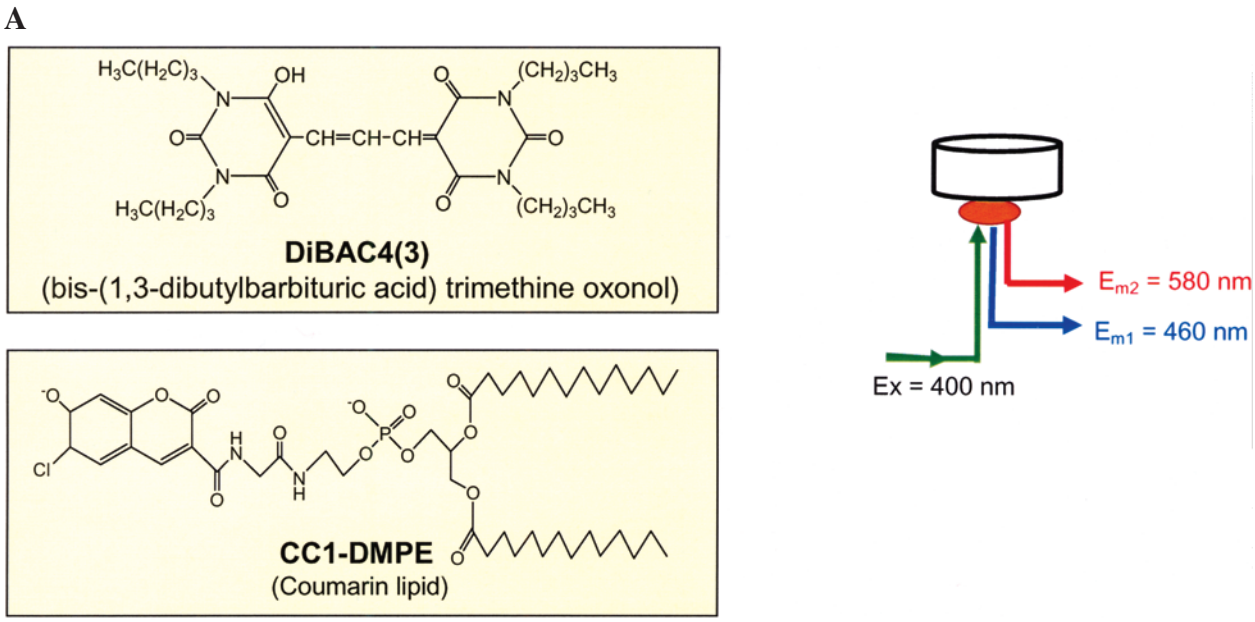

FIG. 2. Schematic illustration of the assay mechanism for FRET-based voltage sensing. (**A**) Structure of the phospholipid-linked FRET donor (coumarin) and FRET acceptor (oxonol). The excitation and emission frequencies of the FRET donor are 400 and 460 nm, respectively, and emission from the FRET acceptor is 580 nm. The emission from both the FRET donor and acceptor are measured simultaneously using the Voltage/Ion Probe Reader (Aurora Discovery, Inc.). (**B**) At the resting membrane potential (approximately −60 mV), fluorescence energy is transferred from the coumarin donor to the oxonol acceptor, resulting in fluorescence at 580 nm. During depolarization, the FRET acceptor (oxonol) translocates to the inner membrane leaflet, resulting in a decrease in FRET emission at 580 nm and a concomitant increase in emission from the FRET acceptor at 460 nm. The relative intensity at 460 and 580 nm provides a readout of changes in the membrane potential of the cell.

quencher(s) absorb the emission of the voltage-sensitive dye when it is positioned in the outer layer of the cell membrane. When the cell membrane is depolarized, the dye moves to the inner layer of cell membrane and upon excitation emits a detectable signal (Fig. 3). The throughput of this assay (in 384-well format) is 60,000–80,000 compounds per day, and the screening cost is relatively high (because of the price of dye kit). A similar membrane potential dye kit, ACT:One, is also available from BD Biosciences (Franklin Lake, NJ).

The homogeneous nature of this assay, combined with the use of a CCD-based imaging instrument that measures the entire plate at once, helps to minimize well-to-well variation. This is important since the signal-to-noise ratio in this assay is low (1.5–2.5). We have utilized this type of an assay for an HTS on a chloride channel in which the signal-to-noise ratio was only 1.4. Despite this small signal-to-noise window, the hit confirmation rate for the HTS screen was 56%.

A direct comparison of this assay with a FRET-based voltage-sensing dye assay on the same channel revealed that the activities of small molecule compounds were less potent in the no-wash assay (W. Zheng *et al.*, unpublished data). We have also observed that the activities of peptides and peptide/protein-based toxins were greatly reduced in the no-wash dye assay but could be detected in the FRET-based dye assay. The noted reduction in activity may be a result of interference from the quencher(s) or other unidentified components in this no-wash dye kit or that the dyes themselves associate not only with the inner layer of cell membrane but also the membranes of subcellular organelles.

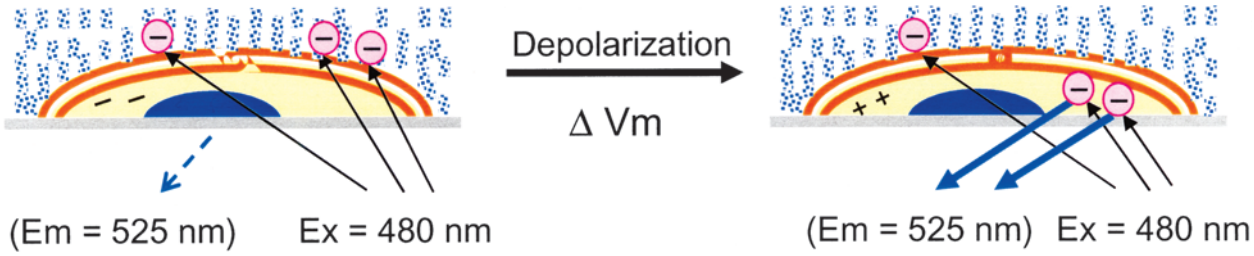

FIG. 3. FLIPR membrane potential assay. A voltage-sensitive dye, together with fluorescence quenchers, is added to cells. The distribution of dye across the membrane is related to the membrane potential. At the resting membrane potential (approximately −60 mV), voltage-sensitive dye molecules are associated with the extracellular leaflet of the cell membrane, where non–membrane-permeable quenchers in the assay buffer diminish their fluorescence. Upon depolarization, dye molecules rapidly translocate into the inner membrane resulting in an increase in fluorescence intensity. The membrane potential dye kit is available from Molecular Devices.

Ion flux assay

Radioactive ion flux assay. Radiotracers can be used to measure the flux of ions moving in or out of a cell via ion channels expressed in the cell membrane. Radiotracers are available for every class of ion channels: $^{86}Rb^{+}$ for potassium channels, $^{22}Na^{+}$ for sodium channels, $^{45}Ca^{2+}$ for calcium channels, and $^{36}Cl^{-}$ for chloride channels. Although these radiotracers have been used for over 20 years in ion channel assays, their application for HTS drug screening has been limited. Tracer assays are heterogeneous and slow, requiring both a tracer loading and wash steps. Only the steady-state function of ion channels can be measured with radiotracers. Signal-to-noise ratio is low because of incomplete removal of extracellular tracer after loading or the continuous leak of tracer out of cells. Concerns over excessive radioactive waste and safety also limit radioactive flux assay use in HTS.

AAS. Ion flux assays using non-radioactive ion tracers analyzed in AAS have been utilized since the 1950s. Commercial instrumentation for high-throughput AAS screening has only become available within the last few years (Fig. 4). AAS assays have been developed for a variety of voltage-gated[22] and ligand-gated channels.[23] For this assay, non-radioactive tracer ions are loaded into cells expressing the channel of interest. Ion flux is then initiated when channels are activated by a ligand or by depolarizing the cell

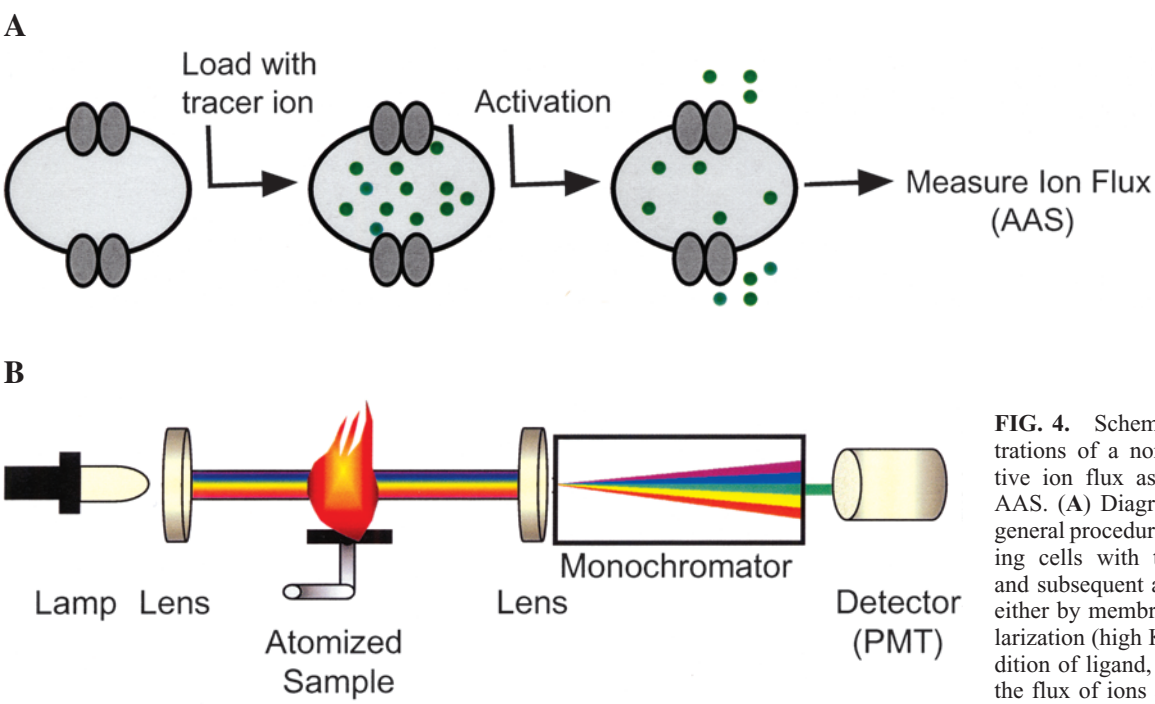

FIG. 4. Schematic illustrations of a non-radioactive ion flux assay using AAS. (**A**) Diagram of the general procedure for loading cells with tracer ion and subsequent activation, either by membrane depolarization (high K^{+}) or addition of ligand, to initiate the flux of ions across the cell membrane. (**B**) Tracer ion concentrations are sensitively quantified using an atomic absorption spectrometer. Vaporized atoms are converted to their ground state within a flame where they absorb light of specific wavelengths. PMT, photomultiplier tube. An automated plate reader for 96/384-well plates is available from Aurora Biomed, Inc. (www.aurorabiomed.com).

membrane with a high K^+ buffer (50–80 m*M*). The concentration of the tracer ion in the supernatant and/or within the cells is then measured, and the percentage of efflux (or influx) is calculated. The signal-to-noise ratio of this assay is typically 6–10, and the reagent cost for screening is very low. Both single-channel and multichannel instruments are currently available for 96-well and 384-well screens with moderate throughput (Aurora Biomed, San Diego). Reports describing the application of this technology for screening assays have been primarily focused on potassium channels such as hERG, KCNQ2, and Ca^{2+}-activated potassium channels where Rb^+ is used as the tracer ion.[24–26]

Electrophysiology

Patch-clamp. Patch-clamp electrophysiology methods are regarded as the gold standard for measurement of compound activity on ion channels *in vitro*. Through an electrode attached to the cell membrane, the current generated by the ions flowing through ion channels expressed in the cell membrane can be measured while the membrane potential is voltage-clamped (Fig. 5). The activity of ion channels is measured directly and in real-time. Despite the high-quality data generated by this method, in its traditional format patch-clamping has limited use in drug screening for ion channel targets because of very low throughput. However, in the past 2 years several automated patch-clamp instruments have been developed and are now commercially available.[28]

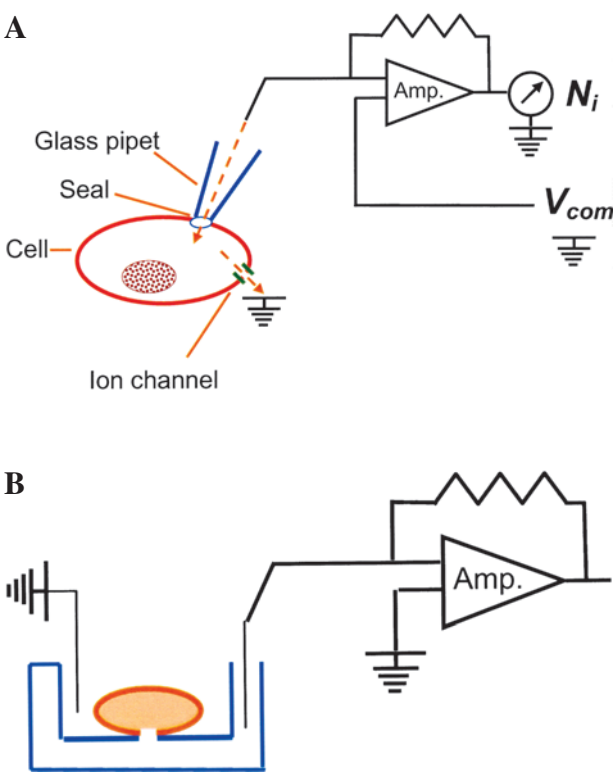

FIG. 5. Schematic illustration of patch-clamp configurations: (**A**) standard micropipette patch-clamp configuration and (**B**) planar chip patch-clamp configuration.[27]

Automated patch-clamp electrophysiology. Traditional patch-clamp electrophysiology incorporates the use of a glass micropipette electrode, microfabricated from glass capillary tubes, for controlling the membrane potential while measuring ionic current flow. The breakthrough that made automating this process for truly higher throughput feasible was the development of the planar patch-clamp electrode. The traditional single micropipette electrode was replaced with a planar substrate with an array of microapertures.[27,28] The first commercially available instrument (IonWorks, Molecular Devices) uses a planar 384-well disposable polyimide plastic plate. In order to perform patch-clamp recordings, cells (in suspension) are first added to electrically isolated wells on the PatchPlate™. Each well on the PatchPlate contains a single aperture for the patch-clamping of a cell. A slight negative pressure is used to pull the cell membrane into the aperture and achieve a 50–600 mΩ seal. Electrical access is achieved via a perforating agent applied at the bottom surface of the PatchPlate. Recent reports show that the pharmacology on several voltage-gated ion channels accurately reflects traditional patch-clamp data. The success rate of patch is between 60% to 90%, and the assay throughput for processing each 384-well plate is ~1 h. From 2,000 to 3,000 cells can be patch-clamped in a day, and 50–100 dose responses can be acquired with this system, representing a 100-fold increase in throughput over the traditional patch-clamp technique.[29,30] The low seal resistance limits the use of this technology to cell lines with robust and homogeneous expression of voltage-gated ion channels.

Another automated planar patch-clamp instrument that recently became commercially available uses a 16-well disposable glass chip (PatchXpress 7000A, Axon/Molecular Devices). As in the traditional patch-clamp, a GΩ seal resistance can be achieved on these chips with a success rate of 20–70%. In this system, electrical access is achieved by rupturing the cell membrane underneath the aperture. Asynchronous operation and the integrated fluidics allow ligand-gated as well as voltage-gated channels to be assayed with this instrument. Pharmacological studies with this instrument demonstrate a good correlation with traditional patch-clamp data. The two- to 10-fold increase in throughput this system offers is attractive for detailed studies of ion channel pharmacology as well as directed screening of small sets of compounds.[31] Ideally, this system fits well alongside the previously discussed 384-well instrument. Whereas the 384-well instrument is utilized to filter through hundreds to thousands of compounds, the 16-well system is used to perform detailed electrophysiological studies on the identified leads. The high cost of consumables will greatly impact the use of these instruments. The

evolution of automated patch-clamp electrophysiology is only in its first stage. Several other automated planar patch-clamp and oocyte clamp systems[32–36] are available or in late-stage development, and the next few years promise to be an exciting time for this technology.

Perspectives for Ion Channel Screening Technologies

Automated patch-clamp electrophysiology

The quality and throughput of automated patch-clamp instrumentation will continue to improve in the next 3–10 years with advances in microfabrication and micromachining technologies for existing/novel planar electrode substrates. This should drive down the cost of consumables and make the technology more readily accessible and widely used for the screening of ion channels. The immediate impact of this technology will be for secondary screening and ion channel safety assessment.

Non-invasive detection of ion channel activity

Current microelectrode based patch-clamp methods are invasive and can disrupt intracellular physiology. Microelectrode array technology is a new approach for non-invasive extracellular recording of ion channel activity.[37] Currently, this technology has been used to record ion channel activity in tissue slices and cultured cardiac cells in single-well/chamber format. Currently, throughput is low, but information content is high. With the miniaturization of microelectrode arrays and the development of multiwell detection, its application for ion channel screening will be further explored. Other label-free detection technologies such as resonant acoustic profiling, microplate differential calorimetry, atomic force microscopy, and microwave spectroscopy may also be developed for non-invasive and high-throughput detection of ion channel functions in the future.

Next generation true high-throughput instrumentation

The current version of automated patch-clamp technology cannot meet the demands of HTS for large compound collections. The fluorescent dye-based and ion flux assays only measure steady-state ion channel activity, which may not reflect the physiological condition of ion channels. Additionally, agonists or toxins are usually required to activate channels in these screens. The next generation of patch-clamp-based screening technologies will have to incorporate even higher throughput without compromising data quality.

Ion channel biology

The pore forming α subunit of an ion channel is made up of multiple subunits. Each class of ion channel also has multiple auxiliary subunits that can modulate the activity of the α subunit. Voltage-gated calcium channels, for example, consist of α_1, α_2–δ, β, and γ subunits, and each of these subunits has multiple isoforms and splice variants. The strategy for cell line generation of ion channels is complicated by the variety of α and auxiliary channel subunits. It is difficult not only to stably transfect all the subunits into a cell line, but also to select the "right" combination of these subunits. Validation of biologically relevant ion channel targets including auxiliary subunit components will continue to be an important area for ion channel drug discovery. In addition, the use of transfected cell lines versus native cell lines for ion channel screening warrants further investigation.

Conclusions

Currently, fluorescence-based assays remain the most frequently used method for the primary screening of large compound collections in ion channel drug discovery. Ion flux and automated patch-clamp assays are the choice for secondary screening and lead optimization. Although the screening throughput and quality have been greatly improved compared to those 10 years ago, ion channel screening technologies need further innovation, refinement, and optimization. Ion channel assays for future HTS will have to be miniaturized into 1,536-well or higher density formats to accommodate the increasing capacity for the screening of multimillion compound libraries. New screening technologies are especially needed for the ion channel targets that cannot be screened with existing technologies because of low channel expression in cells. In addition, more reliable and cost-effective methods are needed for ion channel safety assessment.

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