Unlocking the molecular mechanisms of antipsychotics – a new frontier for discovery

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Summary

Despite the use of antipsychotics to treat schizophrenia for the last several decades, little was understood about their molecular mechanisms of action. In this review, we discuss recent studies that have helped elucidate mechanisms of action of antipsychotics and their potential interplay with genetic, metabolomic, proteomic, and other cellular process-related discoveries in schizophrenia pathology. We also highlight genes that have been identified in multiple studies in both schizophrenia patients and in antipsychotic action that are related to glucose and cellular metabolism, the cytoskeleton, protein synthesis, cell adhesion and synaptic activity. Though some questions of antipsychotic mechanisms of action, such as primary versus off-target effects, remain, the recent gains in understanding how to treat schizophrenia at the molecular level are promising. We propose that these recent insights provide a new and more complete landscape for drug discovery and patient biomarker development.

Key words: antipsychotics; schizophrenia drug development; schizophrenia proteomics; schizophrenia genomics; schizophrenia biomarkers; antipsychotic mechanisms

Antipsychotics – a history

Antipsychotics were discovered in the late 1950s in an effort to improve current anesthetic agents at the time. Though these new agents were ineffective as anesthetics, they robustly attenuated psychotic symptoms in patients, and thus became rapidly popular for their clinical application.

In the 1960s and 70s, researchers determined that this first class of agents, or “typical” antipsychotics, acted primarily on dopamine D2 and related receptors (DRD2). The pharmacological profile of first generation antipsychotics concurred, together with other experimental observations, to the hypothesis that schizophrenia was caused by abnormal dopamine signaling [1–3]. Subsequent studies have revealed irregularities in synaptic function across a broad range of brain regions and in different neurotransmitters in schizophrenia patients, which have led to a clearer, but still incomplete, understanding of the disorder and the drugs that treat it.

With between 0.4% and 1% of the population suffering from schizophrenia in their lifetime [4, 5] and over 3.1 million people being treated with antipsychotics in the United States of America in 2011 alone [6], it remains an important goal to establish the underlying mechanisms of the disorder and its treatments to improve the quality of care and life for schizophrenia patients.

Although antipsychotics were efficacious for positive psychotic symptoms, such as hallucinations, delusions, and paranoia, they remained less effective for the negative (e.g.anhedonia and improper facial affect) and cognitive symptoms associated with schizophrenia [7]. In addition, the first generation of antipsychotics, including haloperidol, were associated with debilitating extrapyramidal side effects following long-term treatment including tremors and Parkinsonism.

In an effort to improve the treatment of the negative and cognitive symptoms and to decrease the side effect profile, “second generation” or “atypical” antipsychotics were introduced in the late 1980s and early 1990s. The atypical antipsychotics continued to target DRD2, but they also targeted other receptor groups such as the serotonin 5HT2A receptors, which are more widely expressed in the hippocampus and other brain regions associated with learning and memory. Atypical antipsychotics were quickly lauded for their improved efficacy and attenuated side effects, and they became the standard of care very quickly. The claims of increased efficacy with fewer side effects of the atypical antipsychotic drugs were increasingly questioned as clinical studies emerged suggesting that, while indeed they did not induce as many extrapyramidal side effects, they were associated with weight gain and obesity in patients [8]. In addition, both typical and atypical antipsychotics presented similar challenges to basic scientists attempting to study cognition in animal models of schizophrenia as, with the exception of clozapine, they induced catalepsy, or “a condition of diminished responsiveness usually characterized by a trancelike state and constantly maintained immobility” [9, 10]. Catalepsy rendered learning and memory studies difficult to interpret, and subsequently interfered in attempts to understand fully antipsychotic actions on cognition.
The question of improved clinical efficacy in treating schizophrenia of atypical versus typical antipsychotics drew further attention in 2005, when multicenter trials revealed no overall amelioration of schizophrenia (both positive/negative symptoms) or side effects between the two groups, with important differences in the types of side effects affected [11]. Extrapyramidal side effects and tremor were more common with typical antipsychotics, while obesity and subsequent diabetes were more common with atypical antipsychotics. Clozapine, which had been previously thought to be favorable for treatment-resistant schizophrenia patients [12], was deemed a potentially promising alternative because of the occurrence of fewer extrapyramidal side effects [13]. However, clozapine usage has also been associated with rare, but severe toxicity, including an increased rate of agranulocytosis, adverse cardiovascular events, seizures, and potentially increased mortality in elderly patients, leading to multiple Boxed Warnings in the US prescribing information. Aripiprazole, another atypical antipsychotic approved in 2002, a partial DRD2 agonist and 5HT2a antagonist, also showed promise, and many also believed its efficacy profile offered improvement for those who could not tolerate the side effects of the other antipsychotics [14, 15]. However, it did not yield a significant improvement in schizophrenia symptoms over risperidone, with conflicting reports on efficacy compared with haloperidol based on treatment duration [15, 16].

Therefore, despite the initial enthusiasm for atypical antipsychotics, further studies revealed that they did not improve efficacy compared with typical antipsychotics, were associated with concerning, albeit different, side effects, and therefore the search for better schizophrenia treatments was renewed.

Because the bulk of new antipsychotics were derivatives of the original discovery, new development efforts searched for a new direction to improve efficacy against both positive and negative symptoms. Many new attempts in the 1990s through the past decade have centered on the hypothesis that schizophrenia results from disrupted glutamatergic signaling (reviewed in [17]). Two such compounds, bitopertin, a glycine transporter type 1 inhibitor that increases glycine in the synaptic cleft, which co-activates N-methyl-D-aspartate receptors (NMDAR) with glutamate, and pomaglumetad, a metabotropic glutamate receptor II agonist were tested in clinical trials; however, both resulted in phase III trial failure, as they did not achieve their primary endpoints [18–20]. With these disappointments in the past few years, there is a need to refocus on understanding the pathophysiology of schizophrenia and the mechanism of action of current antipsychotic drugs. To that end, this review summarizes several recent studies that further elucidate the molecular targets of antipsychotic activity and how they might interface with schizophrenia pathology.

Antipsychotic signaling – what we know now

Although initial studies focused on antipsychotic action at the receptor level, questions remained about the action of antipsychotics at the molecular and signaling levels. Because DRD2 are G-protein coupled receptors Gia type receptors, the primary characterized downstream signaling pathways are adenyl cyclase-cyclic AMP (cAMP) – protein kinase A (PKA) (G-protein dependent cascade) and β-arrestin2 – phosphatase 2A-Akt (G-protein independent cascade).

The majority of antipsychotics block DRD2 receptors and increase cAMP production by removing the tonic inhibition exerted by Gia proteins on adenyl cyclase activity, which depends on the action of the neuromodulator adenosine. The enhanced adenyl cyclase activity increases cAMP levels, activating PKA and increasing PKA-dependent phosphorylation of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of molecular weight 32000) [21–24]. An important functional consequence of the opposite role of DRD2 and adenosine 2A receptors (A2AR) on cAMP production is that genetic or pharmacological blockade of A2AR prevents the ability of DRD2 antagonists (haloperidol, etiolepide, etc.) to increase the cAMP-PKA-DARPP-32 signaling pathway [25, 26]. Antipsychotics acting as DRD2 antagonists also inhibit the assembly and activation of the β-arrestin2 and phosphatase 2A complex. When assembled, this complex dephosphorylates and deactivates Akt (=protein kinase B), and therefore its blockade leads to increased Akt signaling [27, 28].

Despite evidence that PKA and Akt signaling pathways are modulated by multiple first and second generation antipsychotics, the extent of conservation of these mechanisms varies between different antipsychotic drugs. Masri and colleagues noted that the antipsychotics haloperidol, risperidone, ziprasidone clozapine, olanzapine, and aripiprazole all prevented the association of β-arrestin2 to the DRD2 induced by administration of a DRD2R agonist (quinpirole) in a cell culture-based assay [29]. In contrast, the accumulation of cAMP varied between these drugs, suggesting there may be slight variations in the PKA signaling pathway between antipsychotic classes [29]. Because prevention of β-arrestin2/phosphatase2A recruitment leads to the phosphorylation and activation of Akt [28–30], Akt became a strong focus for convergent antipsychotic signaling mechanisms.

The conserved activation of Akt signaling nicely complemented previous work demonstrating that typical antipsychotics lead to phosphorylation of glycogen synthase kinase 3β (GSK3β), altering β-catenin signaling [28, 31, 32]. Interestingly, activation of Akt-GSK3β signaling has also been demonstrated for the newer generation of atypical antipsychotics, such as aripiprazole [33]. Akt-GSK3β action additionally occurs in the different context of mood stabilizers, such as lithium, which also targets DRD2 and GSK3β directly [30]. Therefore, Akt signaling remains the most common and robust downstream signaling effector of the different classes of antipsychotics acting on DRD2. Moreover, activation of Akt is upstream of multiple signaling cascades involved in transcription, protein synthesis, metabolism and other cellular processes. Therefore, the implication of Akt signaling in the mechanism of action in antipsychotics raised the question of whether other cellular processes downstream of Akt might also be involved in antipsychotic action. The Akt-mTORC1 (mechanistic target of rapamycin complex 1)-protein synthesis pathway
was of particular interest as previous studies have shown that long-term antipsychotic treatment alters the proteome in human schizophrenia patients [34]. Recently, Bowling and colleagues demonstrated that acute antipsychotic treatment induces the Akt-mTORC1-protein synthesis pathway resulting in immediate changes in protein synthesis [35]. The two main downstream effectors of mTOR are both upstream of protein synthesis and are 4E-binding protein (4E-BP) and p70 ribosomal S6 kinase 1 (S6K1). Interestingly, other studies have demonstrated that ribosomal protein S6 (Rps6), which has multiple phosphorylation sites that are specific and nonspecific targets of S6K1, can be phosphorylated downstream of PKA signaling as well [21, 36], suggesting some level of convergence on ribosomal signaling following antipsychotic treatment. In addition to the mTORC1 pathway, investigation has also demonstrated an antipsychotic-mediated activation of the Akt-FOXO (forhead box O) pathway [37] suggesting that other downstream Akt effectors may be involved in the antipsychotic signaling as well. Overall, these data indicate a critical role for PKA and Akt signaling in the mechanism of action for antipsychotics (fig. 1).

Animal models
In the past, the most commonly utilized animal models of schizophrenia were centered on the idea that dopamine dysfunction was implicated in this disorder. Indeed, as mentioned earlier in this review, many of the effective antipsychotic drugs were designed as antagonists of dopamine receptors and dopamine agonists may induce symptoms that resemble psychosis. However, the recent discoveries about the pathophysiology of schizophrenia determine the emergence of novel animal models, mimicking some of the clinically relevant human phenotypes. To date, there are four major classes of animal models used to investigate schizophrenia pathology and the efficacy of antipsychotics: genetic models, pharmacological models, neurodevelopmental models, and lesion models [38]. The bulk of publications and effort have focused on genetic and pharmacological models as a means of exploring antipsychotic efficacy and mechanism of action.

Genetic models
Genetic animal models of human disorders are based on the idea that manipulation of the susceptibility genes account for the risk of illness and, together with environmental factors, for phenotypic variation. Schizophrenia is a highly heritable neuropsychiatric disorder that probably involves multiple genes [39]. Targeted manipulations of schizophrenia-associated genes may provide unique advantages in understanding the genetic contribution to the pathophysiology of the disorder and the disease-related endophenotypes. This approach is important to gain knowledge on the molecular pathways, neuronal circuits and behaviors affected in schizophrenia. Moreover, the genetic models allow investigation of interactions between susceptibility genes, the relations between genes and environment, and the effects of genetic manipulation on disease development [40].

We shall describe two such models based on the association between schizophrenia and the candidate susceptibility genes, neuregulin 1 (NRG1) and disrupted-in-schizophrenia 1 (DISC1).

Neuregulin 1 (NRG1)
The genetic association between NRG1 and, to lesser extent, its receptor erbB4, and schizophrenia has been supported in most genetic and meta-analysis studies of various populations [41, 42] including genome-wide association studies [43–45]. Animal models lacking different portions of the Nrg1 gene have been engineered and studied in order to understand the different array of molecular and behavioral phenotypes correlated with the particular deletion. In these models, all the deletions in the Nrg1 gene are in heterozygosis since the homozygote deletions are lethal [41]. Overall, these mice display deficits in prepulse and latent inhibition (PPI and LI, respectively), anxiety, alteration in motor activity and abnormal social behavior [46–49]. Surprisingly, mice with heterozygote deletion of Nrg1 gene have an intact spatial and working memory. The behavioral deficits are also accompanied by changes in neurotransmitter and synaptic receptors, such as decreased expression of NMDA receptors and increased expression of serotonin 2A receptors and serotonin transporter [49]. Importantly, some of the behavioral phenotypes displayed by these mice could be corrected by treatment with the antipsychotic drug, clozapine [41, 46]. Also, transgenic mice with overexpression of the Nrg1 type 1 isoform show deficits in acoustic startle and PPI, hyperactivity and age-dependent disruption of short-term memory [50, 51]. These studies suggest that alteration of Nrg1 gene dosage (de-
leion or overexpression) generates behavioral phenotypes consistent with clinical symptoms of neuropsychiatric disorders.

Moreover, given the important role of Nrg1 in oligodendrocyte development, Roy and colleagues developed transgenic mice expressing a dominant negative form of the gene exclusively in oligodendrocytes [42]. They demonstrated that blocking NRG1-erbB signaling in oligodendrocytes resulted in changes in the number and morphology of oligodendrocyte as well as decreased myelin thickness and reduced conduction velocity in the central nervous system axons. Moreover, these animals displayed anxiety, hypoactivity, and deficit in social behavior. The behavioral and morphological phenotypes were accompanied by increased levels of dopamine receptors and dopamine transporter. This study indicates that NRG1-erbB signaling is important in oligodendrocyte development in vivo and the resulting alteration in brain myelination is correlated with the onset of behaviors consistent with human neuropsychiatric disorders.

Disrupted-in-schizophrenia (DISC1)

Disrupted-in-schizophrenia (DISC1) was initially identified as a gene affected by a translocation mutation that segregated with psychiatric disorders, such as schizophrenia and depression, in a Scottish family [53]. Subsequent studies suggested that variation in DISC1 may play a role in schizophrenia and other psychiatric disorders in normal individuals [54–56].

Mouse models with heterozygote Disc1 deletion were generated to model the translocation mutation discovered in patients [57, 58]. Behaviorally, these mice show higher impulsivity, while locomotor activity and PPI are not different from wild-type littermates. However, the mice display a specific pattern of cognitive impairments, with working memory being severely affected. These cognitive deficits are accompanied by a specific neuronal pathology in the hippocampus, particularly in the neurogenesis process. These studies demonstrated that deletion of Disc1 give rise to phenotypes resembling human neuropsychiatric disorders.

Other models with inducible expression of the human DISC1 gene in specific brain regions, such as cerebral cortex, hippocampus and striatum [59] were produced in accordance with the idea that a mutant truncated DISC1 protein with dominant-negative effects is generated as result of the human translocation mutation. These mice showed an enlargement of the ventricles but no other gross anatomical abnormalities in the brain. Behaviorally, the mice displayed hyperactivity, and deficits in social behavior and spatial memory. Similarly, mice expressing a dominant-negative form of DISC1 [60] present enlargement of the brain ventricles and behavioral abnormalities, including hyperactivity, PPI and affective behaviors. Importantly, these studies suggest that the expression of a truncated non-functional DISC1 protein is associated to anatomical and behavioral changes mimicking human neuropsychiatric disorders.

Animal models with reversible and inducible induction of the C-terminal part of Disc1 [61] were produced based on the demonstration that during development Disc1 is highly expressed in the brain. In these mice, early post-natal Disc1 expression affects synaptic transmission and morphology of neurons in the dentate gyrus. Moreover, this is accompanied by depressive-like behaviors, abnormalities in social behaviors and impairments in working memory. This study suggests that alteration of Disc1 during development may determine the appearance of schizophrenia-like behaviors in the adulthood.

Additionally, RNA interference was utilized to study the function of Disc1 during development. Small hairpin RNA (shRNA)-mediated downregulation of Disc1 during neurogenesis led to morphological and cytological changes in the dentate gyrus, for instance the appearance of neurons with ectopic apical and basal dendrites. Moreover, the neurons showed increased excitability, alteration in the neurogenesis process and aberrant localization of the new-born neurons [62]. These studies underscore the importance of DISC1 expression during early development.

Pharmacology models

While the most popular models for the negative symptoms of schizophrenia are genetic, the most commonly used models for positive symptoms are pharmacological. The two most commonly used paradigms unsurprisingly correspond to the two popularly held neurotransmitter theories of schizophrenia: glutamate and dopamine. For one paradigm, a “schizophrenia-like” state is induced by manipulating NMDA receptors with either phencyclidine or MK-801 and in the other dopamine release is triggered in large quantities by increasing dopamine release with amphetamine or related agents.

MK801/phencyclidine

In the 1950s, phencyclidine was developed as an anesthetic and quickly gained notoriety for causing psychotic-like states in healthy patients. Clinicians noted the similarities between the psychosis induced by phencyclidine and that of schizophrenia patients, and sought to better understand the pathology of psychosis by administering phencyclidine to schizophrenic patients. The administration of phencyclidine led to an exacerbation of symptoms in both stabilized and acute patients, which did not occur with lysergic acid diethylamide (LSD) or other pharmacological agents (reviewed in [63]). These data contributed to the formation of the glutamate hypothesis of schizophrenia and quickly the injection of phencyclidine or another NMDAR antagonist into rodent models to simulate schizophrenia-like behavior. Treatment with this class of inhibitor leads to increased locomotion and altered abnormal sensory-motor gating, and may mimic some of the negative symptoms of schizophrenia such as anhedonia, abnormal social behavior, and decreased cognitive performance on learning tasks (reviewed in [64]). There have been conflicting reports on the ability of typical and atypical antipsychotics to rescue these induced phenotypes [65–67], as well as of newer drugs targeting glycine transporters that increase glutamatergic signaling and glutamate receptor agonists [68–71].

Amphetamine

Amphetamine increases the amount of dopamine in the synaptic cleft and the activity of dopamine receptors. Because the dopamine hypothesis of schizophrenia suggested
increased dopamine signaling, amphetamines and related stimulants were a natural early choice for modeling schizophrenia in rodents. In the amphetamine-based studies, mice were given a dose of amphetamine, which increased locomotor activity, agitation, altered sensory-motor gating and was thought to induce psychotic-like symptoms and behavior including increased locomotion, altered sensory motor gating, and disordered attentional processing (reviewed in [72–76]). Either preceding or following the induction of this altered state where rodents are believed to exhibit “schizophrenia-like” symptoms, animals are given antipsychotics to prevent or correct these behaviors. To date, typical and atypical antipsychotics have been shown to reverse or prevent many of these “positive symptom-like” behavioral deficits; however this is somewhat dependent on the treatment paradigm ([75], reviewed in [38]). The benefit of this model is the induction of “psychosis-related behavior.” However, other studies have contradicted the effect of antipsychotics on these induced “schizophrenia like states,” making them harder to interpret. In addition, the kinetics of the two pharmacological agents – the induction of the symptoms and, separately, the treatment – increase the margin of error and introduce potential differences in pharmacokinetics, pharmacodynamics, signaling changes, and metabolism between the two phases of drug treatment. These potential interactions should be considered when interpreting the data from these models. Many studies have also use wild-type mice to study the mechanisms of action of antipsychotics on circuits at molecular, electrophysiological and behavioral levels, and to predict side effect profiles, which are discussed throughout this review.

Disadvantages and advantages of animal models

Although animal models have been useful in probing mechanistic questions of antipsychotics and side effects, they have had limited benefit in predicting efficacy. There are many complex reasons that the lack of translation from animal models to the patient population may occur. This lack of efficacy prediction could be the result of over-extrapolation of the behaviors, as mice are less cognitively complex as humans, or it could be related to an over interpretation of efficacy. In addition the human schizophrenia population is diverse with no single unifying environmental or genetic known cause, which could lead to subgroups that respond differently to drugs, whereas mouse models are comparatively a very homogenous population. These differences between animal models and humans complicate both preclinical studies and clinical trial design and interpretation; however, when used with caution, animal models can be informative of mechanism and side effect profiles. Many researchers have also noted that part of the issue with designing better animal models is that the pathology in humans is so poorly understood and unbiased metrics are lacking for research and clinical trials [77]. In summary, there are many benefits to using different schizophrenia animal models to understand the mechanism of action for antipsychotics, but the field should remain cautious in directly extrapolating animal model data to human patients without further confirmatory studies.

Antipsychotics and “omics”: additional insights from transcriptomic and proteomic studies

While these recent molecular signaling cascade studies provide important insights into the pathways that are immediately affected, how these molecular shifts lead to antipsychotic action at the cellular, circuit and behavioral level remains unclear. Previous clinical research has shown that though antipsychotic action can take up to 3 weeks to reach fully efficacy, some symptom improvement is noted within the first 24 hours of antipsychotic treatment [78, 79]. To better understand how the fast-acting molecular changes ultimately incite prolonged antipsychotic efficacy, transcriptomics and proteomics studies have been employed to offer insight into the establishment of long-term changes in neuronal processes (summarized in table 1). Interestingly, transcriptomic and proteomic studies in both human patients and rodents have highlighted a series of convergent targets suggesting that specific cellular processes and transcripts may play a role in antipsychotic action (table 1). One such cellular process, energy metabolism, has been proposed to be a target of antipsychotic action by both acute and chronic antipsychotic studies in both humans and rodents. Changes in the levels of metabolism-related transcripts and proteins, such as DISC1, optic atrophy 1 (OPA1), glutamate dehydrogenase 1 (GLUD1), and AKT1 were regulated by acute and chronic antipsychotic treatment [34, 35, 38–82]. In a 2009 study, Ji and colleagues examined the proteome of cortical synaptoneurosomes of rats treated with antipsychotics for 34 days and noted altered expression of proteins involved in glycolysis and glucose metabolism, further suggesting altered mitochondrial and energy metabolism in the brain following prolonged antipsychotic treatment [83].

The role of metabolic changes was also underscored in a recent study performed on peripheral blood mononuclear cells of schizophrenia patients sampled before and after 6 to 8 weeks of antipsychotic treatment [84]. Gene expression profiling revealed that antipsychotic treatment led to changes in proteins involved in different forms of metabolism, including carbohydrate, amino acid and nucleic acid metabolism. In addition, they demonstrated aberrant transcript abundance of AKT1 and DISC1 in mononuclear patient cells as compared with healthy volunteers before antipsychotic treatment. Interestingly, antipsychotic treatment normalized AKT1 but not DISC1 transcript levels, suggesting that AKT1 may be a stronger antipsychotic target in peripheral tissues.

In the same study, antipsychotics also restored physiologic levels of ribosomal proteins and a transfer RNA (tRNA) synthesizing protein in schizophrenia patients [84]. Consistently, another study also indicated an antipsychotic-driven change in the level of the RNA helicase Ddx5, a translation-related molecule that has been implicated in protein synthesis initiation [85]. Translational proteins in the form of ribosomal proteins were additionally implicated in a study in mice treated with antipsychotics for 2 weeks, as treatment increased the mRNA expression of two ribosomal proteins [86]. Other studies have also noted the upregulation of mTOR and Rps6 transcripts in rodents fol-
lowing antipsychotic treatment [87], which is especially remarkable as ribosomal proteins are thought to be mTOR-specific targets [88], and underscores the evidence of the role of mTOR signaling in antipsychotic action. The upregulation of proteins involved in protein synthesis, such as ribosomal protein and tRNAs, suggest another interesting cellular process that is targeted by antipsychotics. Another piece of evidence supporting the role of changes in protein synthesis (and potentially transcription as well) is data by Bowling et al. [35] that suggest that antipsychotics induce mTORC1 signaling and subsequent changes in ribosomal proteins within the first few hours of treatment and that this effect is maintained for at least 48 hours. Following the initial increase in protein synthesis related proteins such as ribosomal, chaperone and tRNA-related proteins, there was a later phase of increased cytoskeletal proteins. Interestingly, many of these candidate proteins overlapped with the previous reports in rodents and patients who had undergone prolonged antipsychotic treatment, suggesting that cytoskeletal protein expression may be induced within a day of treatment and maintained. One such previously reported cytoskeletal protein was microtubule-associated protein 2 (MAP2). It has been shown that Map2 transcripts and protein levels were increased in the rodent cortex after prolonged (3-week) treatment with antipsychotics [82, 89]. Additional cytoskeletal proteins other than Map2 were identified as upregulated following antipsychotic treatment in rodents and rodent neural progenitor cells (NPCs), including dynamin 1 (Dnm1) and glial fibrillary acidic protein (Gfap) [81, 82]. In summary, transcriptomic and proteomic studies support a role of cytoskeletal proteins following long-term antipsychotic treatment, especially for Map2. In addition to the identified changes in proteins relating to the cytoskeleton, several large-scale studies also showed alterations in abundance of cell-adhesion and synaptic-activity proteins. Chan et al. [34] examined cortices of human schizophrenia patients treated with antipsychotics and compared their proteomes with those of human control subjects. They identified multiple altered proteins, most notably neural cell adhesion molecule 1 (NCAM1) and synaptic-associated protein, 91kDa (SNAP91), which are involved in cell adhesion and clathrin-dependent pre-synaptic vesicle assembly. Synaptic activity proteins Snap91 and synapsin 1 (Syn1) were also increased in proteomic rodent brain and neuron in culture-based antipsychotic studies [35, 82]. The changes in the abundance of proteins associated with metabolism, protein synthesis, cytoskeleton, cell adhesion and synaptic activity across multiple independent studies (table 1) suggest critical roles for these cellular processes in the action of antipsychotics.

Mechanisms underlying disease versus treatment

The wealth of new information on the molecular basis of antipsychotic action provided a more complete picture as to how antipsychotics modulate cellular outputs and processes. However, the question remained of how antipsychotics could be addressing underlying schizophrenia pathology. Several studies in humans, human cell culture systems and animal models have been performed to elucidate some of the potential mechanisms underlying schizophrenia pathology. Many of the studies highlight deficits in regulation in the genes and proteins targeted by schizophrenia that we have summarized in table 2. There are multiple lines of evidence suggesting a disruption in metabolism that nicely compliment the data on antipsychotic mechanism of action. For instance, genetic studies of mitochondrial proteins have noted decreased expression of OPA1 [90, 91] that correlate to increased schizophrenia risk. In addition, increased glutamine dehydrogenase activity (including that of both GLUD1 and GLUD2) has been noted in the prefrontal cortex of schizophrenia patients compared with healthy controls [92]. GLUD1 is basally more active than GLUD2 [93], and likely contributes towards this increased activity [92]. Mitochondria-associated oxidative stress and damage were also evident in NPCs derived from human patient induced pluripotent stem cells (hiPSCs) with increased oxidative stress, decreased mitochondrial size, and altered cellular distribution [94]. Mitochondrial dysfunction, altered metabolism and oxidative stress were again noted in a separate metabolomic and proteomic study in human prefrontal cortex tissue [95]. Metabolic dysfunction was also observed to be a common thread in multiple studies of human schizophrenia patient tissue [96]. Given that antipsychotics have been shown to alter transcription and protein abundance of several of the specific genes indicated in mitochondrial and metabolism defects, the cellular processes regulating metabolism appears to be a site of interplay between known schizophrenia pathology and the action of antipsychotics. In addition to metabolism changes, genes encoding for signaling molecules involved in metabolism and other key cellular processes such as AKT1, DISC1, GSK3β and genes encoding for proteins regulating the mTOR pathway have been implicated in schizophrenia risk and pathology. Although not all populations appear to have a correlation between variants in the AKT1 gene and schizophrenia, several studies across the world have reported AKT1 as a risk gene for schizophrenia [31, 97–101]. Schizophrenia has also been associated with genetic disruptions such as in the DISC1 gene identified in smaller cohort of patients [102]. DISC1 protein, which is involved in the regulation of mitochondrial and cytoskeletal function, has also been shown to interact with Akt-mTOR signaling in mice [103], further suggesting a convergence of signaling in schizophrenia pathology. Indeed, a single nucleotide polymorphism (SNP) in RAPTOR, encoding for the protein interacting with mTOR in mTORC1, has also been found to be predictive of adverse effects in patients who develop extrapyramidal symptoms (EPS) [104]. Protein synthesis and ribosomal proteins, which are also downstream of Akt-mTORC1, have additionally been reported to be downregulated in olfactory cells of schizophrenia patients [105]. GSK3β, another downstream effector of AKT1, also has variations associated with increased risk for schizophrenia in the Han Chinese population [106], suggesting another point of interaction of the signaling cascades implicated in both pathology and antipsychotic action. Because these signaling molecules have been heavily implicated in multiple cellular processes including metabolism, cytoskeleton, pro-
tein synthesis and transcription, they also represent an important point of convergence for pathology and mechanism of action of antipsychotics at the protein and signaling levels.

### Table 1: Cellular processes that are affected in schizophrenia patients and targeted by antipsychotics. Cellular processes with at two or more genes and multiple publications selecting their involvement in both schizophrenia pathology and antipsychotic action.

<table>
<thead>
<tr>
<th>Cellular process class</th>
<th>Reported genes and cellular processes</th>
<th>Gene/RNA/protein</th>
<th>Schizophrenia pathology?</th>
<th>Antipsychotic action?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td>AMPK, GLUD1, DISC1, AKT1, MTOR, OPA1</td>
<td>Gene, RNA, protein Patients and animal models</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Glucose metabolism, ATP synthesis, response to insulin signaling, amino acids, ATP/AMP ratio, glycolysis – gluconeogenesis pathway disruptions, increased reactive oxygen species</td>
<td></td>
<td>Human genetic screen, proteomic data in human patient iPSC NPCs, human metaboliom and proteomic screens</td>
<td>Microarray in human patient blood following treatment, Proteomic data in cultured rodent neurons following treatment, AMPK and mTOR signaling transcripts unbalanced in blood from patients with EPS symptoms</td>
</tr>
<tr>
<td>Protein synthesis and processing</td>
<td>mTOR, RPS6, EEF1A2, ribosomal protein, CCT, RAPTOR, DDX5</td>
<td>Gene, RNA, protein Patients and animal models</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Translation initiation, translation elongation, protein folding</td>
<td></td>
<td>Transcripts reduced in patients compared to control subjects in blood cells, raptor SNP part of risk group for EPS in patients</td>
<td>Microarrays in human patient blood following treatment, one suggests difference between patients with and without EPS, proteomic data in cultured rodent neurons following treatment, microarray data in mouse brain following treatment</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>MAP2, DISC1, DNM1</td>
<td>Gene, RNA, protein Patients and animal models</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Microtubule dynamics, dendritic branching, spine dynamics</td>
<td></td>
<td>Human genetic screen, microarray and proteomic data in human iPSC NPCs, protein levels in human patient brains</td>
<td>Proteomic data in cultured rodent neurons following treatment, increase in transcripts in rodent brains following antipsychotic treatment</td>
</tr>
<tr>
<td>Cell adhesion</td>
<td>NCAM1, CNTN4, NRXN1</td>
<td>Gene, RNA, protein Patients and animal models</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Cell adhesion molecules, synaptic stability</td>
<td></td>
<td>Human genetic screen, microarray and proteomic data in human patient iPSC NPCs</td>
<td>Proteomic data in cultured rodent neurons and human patient brains following treatment</td>
</tr>
<tr>
<td>Synaptic Activity</td>
<td>DISC1, SNAP91, SYN1, BILIN1</td>
<td>Gene, RNA, protein Patients and animal models</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Synaptic transmission, catecholamine synthesis and release, postsynaptic synaptic scaffolding, vesicle recycling</td>
<td></td>
<td>Human genetic screen, microarray and proteomic data in human patient iPSC NPCs</td>
<td>Proteomic data in cultured rodent neurons and rodent brains following treatment</td>
</tr>
</tbody>
</table>

AMPK = 5' AMP-activated protein kinase; EPS = extrapyramidal symptoms; iPSC = induced pluripotent stem cell; mTOR = mechanistic target of rapamycin; NPC = neural progenitor cell; SNP = single nucleotide polymorphism
Table 2: Targets associated with both schizophrenia pathology and antipsychotic action. The following genes have been implicated in at least three studies in both schizophrenia pathology and the mechanism of action of antipsychotics at the genetic, transcript and/or protein level.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Identified at the genomic/ transcript/ protein/ signaling level?</th>
<th>Human or rodent studies?</th>
<th>Implicated in schizophrenia?</th>
<th>Implicated in antipsychotic action?</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>Gene, signaling</td>
<td>Human Rodent</td>
<td>SNPs correlate with schizophrenia risk, reduced AKT1 protein levels in patient brains, one of the SNPs in a cluster that may predict patient EPS risk</td>
<td>Increased signaling in response to acute treatment in rodent neurons, part of DISC1 regulation of neuronal development</td>
<td>Bajestan et al. 2006 [86], Emamian et al. 2004 [31], Kim et al. 2009 [103], Mas et al. 2015 [104], Beaulieu et al. 2004 [50], Bowling et al. 2014 [35], Schwab et al. 2005 [97]</td>
</tr>
<tr>
<td>BIN1</td>
<td>Gene, proteome</td>
<td>Human Rodent</td>
<td>Rare insertion variants in human patients, altered in human patient iPSC NPCs</td>
<td>Upregulated in rat brain proteome following chronic antipsychotic treatment</td>
<td>Tam et al. 2010 [14], Ma et al. 2009 [82], Brennand et al. 2015 [84]</td>
</tr>
<tr>
<td>DDX5</td>
<td>Transcript, proteome</td>
<td>Human Rodent</td>
<td>Measured as altered in human patient iPSC NPCs</td>
<td>Transcripts differentially regulated in EPS vs non-EPS mice treated with antipsychotics, altered in rodent neurons at the proteome level following antipsychotic treatment</td>
<td>Mas et al. 2015 [85], Brennand et al. 2015 [84], Bowling et al. 2014 [35]</td>
</tr>
<tr>
<td>DNM1</td>
<td>Proteome</td>
<td>Human Rodent</td>
<td>Increased in patient brains (proteome)</td>
<td>Increased at proteome level following antipsychotic treatment in rodents</td>
<td>Ma et al. 2009 [82], Prabakaran et al. 2004 [85], Clark et al. 2006 [111], Pennington et al. 2007 [112]</td>
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<tr>
<td>GLUD1</td>
<td>Transcript, Proteome, Enzymatic activity</td>
<td>Human Rodent</td>
<td>Increased glutamate dehydrogenase activity in human patient prefrontal cortex, differentially regulated in human patient iPSC NPCs</td>
<td>Altered in response to antipsychotic treatment in rodents and in patients.</td>
<td>Burbaeva et al. 2003 [92], Ma et al. 2009 [82], Brennand et al. 2015 [84], Bowling et al. 2014 [35], Chan et al. 2011 [34]</td>
</tr>
<tr>
<td>GSK3B</td>
<td>Gene, signaling</td>
<td>Human Rodent</td>
<td>SNPs are a risk factor for schizophrenia</td>
<td>Signaling changes following antipsychotic treatment</td>
<td>Emamian et al. 2004 [31], Beaulieu et al. 2011 [30], Chen et al. 2015 [106], Li et al. 2007 [32]</td>
</tr>
<tr>
<td>MTOR</td>
<td>Gene, transcript, signaling</td>
<td>Human Rodent</td>
<td>SNP of mTORC1 member Raptor indicated as risk factor for EPS in patients in concert with other SNPs</td>
<td>Signaling increased following acute antipsychotic treatment, altered at the transcript level in patient blood following antipsychotic treatment with transcript differences between EPS and non-EPS patients in blood</td>
<td>Mas et al. 2015 [85], Mas et al. 2015 [104], Mas et al. 2015 [126], Bowling et al. 2014 [35], Korostynski et al. 2013 [31]</td>
</tr>
<tr>
<td>NCAM1</td>
<td>Gene, transcript, proteome</td>
<td>Human Rodent</td>
<td>SNPs are a risk factor for schizophrenia, change in abundance in cerebral spinal fluid in patients, changes in abundance in patient serum</td>
<td>Changes at transcript and proteome level following antipsychotic treatment</td>
<td>Atz et al. 2007 [119], Sullivan et al. 2007 [123], Ayalew et al. 2012 [124], Tanaka et al. 2007 [120], Vawter et al. 2001 [121], Pollorak et al. 1997 [2011], Chan et al. 2011 [34]</td>
</tr>
</tbody>
</table>
The next category of cellular processes that is a target of antipsychotic action is the cytoskeleton. MAP2, which was upregulated following antipsychotic exposure in multiple studies, has been overwhelmingly reported to be downregulated in the brains of human schizophrenia patients [107–110]. DNMT1, another cytoskeleton protein, was shown to be increased in patient brains at the proteomic level [95, 111, 112] and was also targeted by antipsychotics [35, 82]. These data firmly support a role of cytoskeletal proteins in both schizophrenia and in its treatment. Because of the hypotheses that schizophrenia is caused by alterations in neurotransmitter signal transduction and synaptic activity, proteins that affect synaptic activity, stability and function have long been an area of intense investigation in schizophrenia. Recently, it has been shown that evoked synaptic release of catecholamines (dopamine, epinephrine and norepinephrine) from hiPSCs was different in schizophrenia and control patients [113]. Not only did the schizophrenia patient-derived neurons exhibit increased catecholamine release, but also proportionally more neurons had proteins to synthesize catecholamines. This suggests that there may be increased catecholamine synthesis and improper increased release in human patients under specific evoked conditions. In addition, they also discovered variations in genes that encode proteins involved in synaptic endocytosis, release and synaptic transmission such as bridging integrator 1 (BIN1) [114], SYN1 [115], contactin 4 (CNTN4) [116, 117] as well as single nucleotide polymorphisms in cell adhesion molecule NCAM1 [119, 123] and exonic deletions in Neurexin 1 (NRXN1) [118]. Moreover, the changes in the cell adhesion molecule NCAM1 in human patient cerebral spinal fluid and serum [119–124] are important because NCAM1 is also a known target of antipsychotic action. Though more investigation is needed, changes in synaptic release proteins, cell-cell interactions and synaptic properties have been reported in patients and by other investigators following antipsychotic treatment [34, 35, 82, 94, 115, 125], therefore, the idea that antipsychotics may correct schizophrenia pathology by decreasing the effects of overly secreted catecholamines and altering the synaptic landscape by changing cell adhesion molecules is potentially promising and merits further investigation.

### Primary versus off-target effects

Given the new insights gained in the potential molecular mechanisms of action of antipsychotics, one important question still remains: how many of these molecular changes are related to the efficacy of treatment versus the appearance of undesirable side effects, such as extrapyramidal symptoms (EPS) and potential neurotoxicity? Though this is an emerging area of research, some studies suggest that this relationship may be complex. Recently, it was discovered that the presence of four SNPs in gene encoding for proteins regulating the Akt-mTORC1 pathway in schizophrenia patients (including AKT1 and mTORC1 member, RAPTOR) predicted an increased risk for EPS after 15 days of treatment [104]. Patients who did not experience EPS had altered abundance of mRNA transcripts related to protein folding in their peripheral blood. In contrast, patients with EPS had changes in transcripts related to mTOR and 5’ AMP-activated protein kinase (AMPK), suggesting a different role of mTOR balance and energy signaling in the onset of EPS [126]. Although these data were not confirmed at the protein or signaling level, taken together, they suggest that changes in the Akt-mTOR pathway may predict the likelihood of EPS [126]. These findings provide evidence that there may discreet signaling differences between EPS and non-EPS patients that could be characterized for biomarkers.

To fully understand the relationship between the Akt-mTORC1 pathway and extrapyramidal symptoms, studies in animal models are required. Indeed, the relationship between mTOR signaling and EPS was investigated in the brains of two rodent strains with different sensitivities to EPS, and it was discovered that the strain with increased sensitivity to EPS had reduced phosphorylation in the mTORC1-dependent Rps6 site, and increased phos-
phorylation in the site that has also been shown to be down-
stream of MEK-ERK and RSK signaling [126–128].

Another preliminary unpublished study discussed by Chao
and Klann [129], found that disruptions in the mTORC1
pathway did not prevent the onset of catalepsy following
acute (within 2 hours of) antipsychotic treatment. Because
catalepsy in animal models has been reported to predict
EPS liability in humans [130], these data further suggest
that the normal activity of the mTORC1 pathway may not
be critical to the onset of EPS following antipsychotic
treatment, but disruptions in mTOR signaling may influence
EPS liability. Indeed, multiple studies have suggested that
molecular signaling pathways other than mTOR contribute
to antipsychotic-induced catalepsy, and thus, potentially
EPS. Many of these pathways are associated with PKA that is a
key regulator of dopamine signaling in the striatum [22].
PKA-cFos signaling and delta FosB have both been re-
ported to be upregulated following antipsychotic treatment
and catalepsy induction [131–133]. Another downstream
effector of PKA, Darpp32 has also been implicated in the
induction of catalepsy as DARPP32 knockout mice have
reduced catalepsy following antipsychotic treatment [134].
Muscarinic receptors have additionally been suggested to
play a role in catalepsy induction as acetylcholine muscar-
inic receptor 4 (M4) knockout mice have diminished cata-
lepsy as well [135]. These studies support a role for signal-
ing pathways other than mTORC1 in the onset of catalepsy,
which may be a predictor for EPS in human patients.
These findings clearly demonstrate a role of multiple sig-
na ling pathways in the induction of extrapyramidal symp-
toms following antipsychotic treatment. Although some
pathways, such as those in the PKA intracellular signaling
cascade suggest a convergent role in EPS, other findings
such as the potential role of atypical mTORC1 signaling
remain under characterized. However, the upregulation of
PKA and aberrant or underactive mTOR signaling may
play a role in EPS in patients and may indicate a potential
area for future biomarkers of EPS.

Biomarkers for patient stratification

The potentially predictive markers for the induction of EPS
in humans and rodents signify possibilities for biomarkers
that could stratify the patient population. As there is no
known unifying genetic or environmental cause of schizo-
phrenia, stratification could both streamline clinical trials
and improve care for patients, as those at risk of developing
EPS could be identified even if it is after beginning treat-
ment but before the development of full EPS. Though both
PKA and Akt-mTORC1 signaling may be promising for
developing biological and unbiased means of patient strat-
fication and EPS biomarkers, they still require more invest-
igation. In addition, the fact that mTORC1 and translation-
related proteins and signaling have been positively corre-
related with efficacy of antipsychotics, and dysregulation
with their side effects, may suggest that careful titration of
these pathways is supremely important in separating effic-
cy from undesirable off-target drug actions. In summary,
the idea of distinguishing which of these newly identified
molecular candidates is important for efficacy versus side
effects (and if this role is temporally dependent) will be an
important focus for future research.

Biomarkers for efficacy

Another hurdle that identifying molecular markers for ant-
ipsychotic action could help overcome is that of unbiased
markers of efficacy. To date, the majority of clinical trials
have relied on rating scales and not unbiased biological
metrics to assess antipsychotic efficacy; however, these
scales can be problematic, difficult to interpret, limited by
erater reliability and their reporting can introduce bias [136,
137]. Therefore, an unbiased biologically based metric per-
fomed either through blood tests, positron emission tomo-
ography, or magnetic resonance imaging or through peri-
teral tissue would be beneficial in assessing the efficacy
of treatment for further trial efforts in most psychiatric and
neuroscience based indications.
The identification of key molecular pathways and players
in efficacious treatment of schizophrenia would not only
open a new avenue for drug discovery, but would also
provide a launching pad for identifying markers of efficacy
that could be used to better design clinical trials. Given that
AKT1 and RPS25 transcripts have been shown to be re-
cued in blood cells following 6 to 8 weeks of antipsychotic
treatment [84], blood-based efficacy markers do seem pos-
sible. Though we have mentioned some key genes, proteins
and cell signaling cascades that could prove an informed
starting point, more careful examination of their relation-
ship with antipsychotic efficacy in specific temporal inter-
vals would be required to fully establish any of them as true
biomarkers. Although many novel targets associated with
antipsychotic efficacy have emerged following recent re-
search, it is important that they be put into the context of
known genetic mutations and proteomic differences associ-
ated with schizophrenia patients (table 2).

Conclusion

Given the wealth of new data in the past decade, there is
now a clearer framework for the mechanisms of action of
antipsychotics and how they could interact with schizo-
phrenia pathology (tables 1–2, fig. 2). Although studies
need to be performed to establish primary versus off-target
effects, this is far more possible than it was a decade ago. Future efforts should focus primarily on (a) elucidating the role of these targets in efficacy vs side effects (may not be direct, but dose or temporally related) (b) identifying biomarkers as a means of tracking efficacy in an unbiased manner for more expedient and clear clinical trials and (c) drug discovery efforts for drugs with more improvements and fewer side effects. With these advances in our understanding, there is renewed optimism for future drug discovery and biomarker efforts.

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Figure 1
Schematic representing the action of antipsychotics on intracellular signaling cascade.
Inhibition of D2Rs by antipsychotic results in increased cAMP levels produced by removing the tonic inhibition on adenylyl cyclase activity (see text for more details). This leads to activation of PKA and phosphorylation of DARPP-32. Moreover, antipsychotics, by antagonizing D2Rs, inhibit the activation of β-arrestin2/PP2A complex resulting in increased AKT activity. Both AKT and PKA lead to activation of mTORC1 and S6 ribosomal proteins, which regulated protein synthesis.

AKT = protein kinase B; cAMP = cyclic AMP; D2R = dopamine 2 receptor; DARPP-32 = dopamine- and cAMP-regulated phosphoprotein of molecular weight 32,000; mTORC1 = mechanistic target of rapamycin complex 1; PKA = protein kinase A; PP2A = phosphatase 2A; S6K1 = p70 ribosomal S6 kinase 1; S6rp = S6 ribosomal protein; TSC = tuberous sclerosis complex.
Figure 2
Schematic representation of reported changes to neurons in schizophrenia (A) and following antipsychotic treatment (B). Organelles are drawn to scale.