addiction (withdrawal-negative affect stage) continues to gain preclinical and clinical experimental support. The endogenous kappa opioid peptides derived from prodynorphin encode the dysphoric, anxiogenic, and cognitive disrupting responses to behavioral stress exposure (Bruchas *et al*, 2010; Carroll and Carlezon, 2013). Drugs of abuse are also profound activators of the brain stress systems, and dynorphin release following a binge of consumption contributes to the dysphoric and anhedonic responses experienced during withdrawal (Koob *et al*, 2014). Behavioral studies using rodents in multiple laboratories have now consistently demonstrated that kappa antagonists do not block the 'euphoric-like' effects of drugs but rather block the stress-induced potentiation of drug reward, block stress-induced reinstatement of drug seeking behavior, and block escalation of drug consumption in long-access models (Whitfield *et al*, 2015). We predict that kappa antagonists will promote stress resilience and disrupt the addiction cycle by reducing the dysphoria-driven cravings that trigger a subsequent round of drug seeking.

However, very exciting preclinical findings too often fail to deliver on their promises, particularly in CNS drug development, which is notoriously expensive and difficult. Progress is being made with a kappa antagonist (LY2456302) developed by Eli Lilly scientists, which passed initial safety testing and has been licensed for development by Cerecor (Lowe *et al*, 2014). Another key to this translational effort will be the further development of selective kappa opioid PET imaging in normal and affected human subjects, which is still at a nascent stage. A more 'out of the box approach' is to take advantage of "creative" pharmacology. Buprenorphine is not only a mu partial agonist, but is a potent kappa antagonist having antidepressant activity (Karp *et al*, 2014). A recent open label clinical trial by Alkermes demonstrated that the nonselective KOR antagonist buprenorphine when combined with a mu opioid antagonist significantly reduced depressive symptoms in a population of individuals having treatment resistant depression (E. Ehrich, Kappa-2015 conference proceedings). Dr Andrew Saxon (Seattle, VA) also reported results from the NIDA-funded CURB study, which showed that while cocaine consumption was not significantly reduced by buprenorphine combined with a long acting mu antagonist, secondary analysis of the data from cocaine-using subjects showed a highly significant reduction in nicotine and ethanol use. Additional, recent findings in the dynorphin-kappa domain reported at the '*3rd Conference on the Therapeutic Potential of Kappa Opioids in Pain and Addiction*' can be found at (http://depts.washington.edu/nidactr/kappatherapeutics2015.html).

Human laboratory studies are an efficient means of bridging the gap between preclinical studies and clinical trials, and we encourage additional validations using more selective kappa antagonists, nevertheless, these early findings are provocative. In summary, the initial results using animal models of psychiatric diseases followed by early validation in human trials support the prediction that individuals unable to control their drug consumption because of overwhelming feelings of dysphoria or anxiety during the abstinence phase, may find kappa antagonists helpful by promoting stress resilience.

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RiboTag: Not Lost in Translation

Measuring RNA from a defined subset of cells derived from a complex tissue is an important challenge that has confounded the field. Two recently developed tools have simplified this issue. The RiboTag and BacTRAP (Translating Ribosome Affinity Purification) methods allow for immunoprecipitation of ribosome-associated RNA from specific cells within complex tissues by expressing tagged ribosomal protein in desired cell types (GFP-tagged RPL10 for TRAP and hemagglutinin-tagged RPL22 for RiboTag) (Doyle *et al*, 2008; Heiman *et al*, 2008; Sanz *et al*, 2009). More specifically, these methods allow analysis of the 'translatome'—ribosome-associated mRNA—which may be particularly sensitive to event-dependent regulation of protein translation. For example, RiboTag-expressing transgenic mice were recently used to compare differential gene expression responses to cocaine in striatal neurons expressing D_1 and D_2 dopamine receptors (Chandra *et al*, 2015).

Because RiboTag and BacTRAP allow the measurement of ribosome-associated mRNA, it is not a direct reflection of the pool of total RNA but can provide intriguing insights into regulatory shifts in protein synthesis in response to a stimulus. Conditional expression using either Cre-dependent viral vectors or transgenic breeding strategies allows highly specific expression of RiboTag in desired cells, and the RNA enriched from the immunopurification can be efficiently harvested and interrogated using RT-PCR, microarrays, or RNA-seq. For example, we have used dual-virus transduction approaches to analyze the molecular phenotype of distinct output pathways in lateral habenula and have utilized transgenic mice that express RiboTag in Pet1-positive neurons to investigate how stress alters the translatome in serotonin neurons.

Recently, our laboratory demonstrated a novel application of RiboTag for *in vitro* cell culture studies (Lesiak *et al*, 2015) (Figure 1). Primary neuronal cultures consisting of both neurons and glia are a common model system for investigating gene regulation and cell physiology, but low transfection efficiency and the presence of mixed cell types complicate detection of experimental manipulations on RNA dynamics. By co-transfecting RiboTag plasmid with experimental plasmids such as DREADD receptors or shRNA-knockdown constructs, we were able to measure changes in gene translation specifically in transfected neurons. These experimental plasmid manipulations can utilize knockdown or overexpression of various proteins and circumvent the problem of RNA contamination from untransfected neurons or glia. For example, co-transfection of RiboTag with wild-type or mutated receptors into cells from a knockout background allows efficient comparison of the effects of a variety of receptor mutants and pharmacological treatments in primary neurons even after sparse transfection. Using cell type-specific promoters to drive RiboTag expression can provide further refinement in translation analysis despite culturing these cells within a more diverse population of primary neurons and glia (Figure 1c and d).

Whether *in vivo* or *in vitro*, the RiboTag strategy provides a new ability to identify altered patterns of RNA translation in specific cell types within a heterogenous background. Although many techniques to measure cell-specific transcription like DROP-seq and FACS require extensive tissue processing and specialized equipment, the RiboTag method is highly flexible, requires minimal specialized equipment, and can be easily established in almost any laboratory. Application of this novel and widely

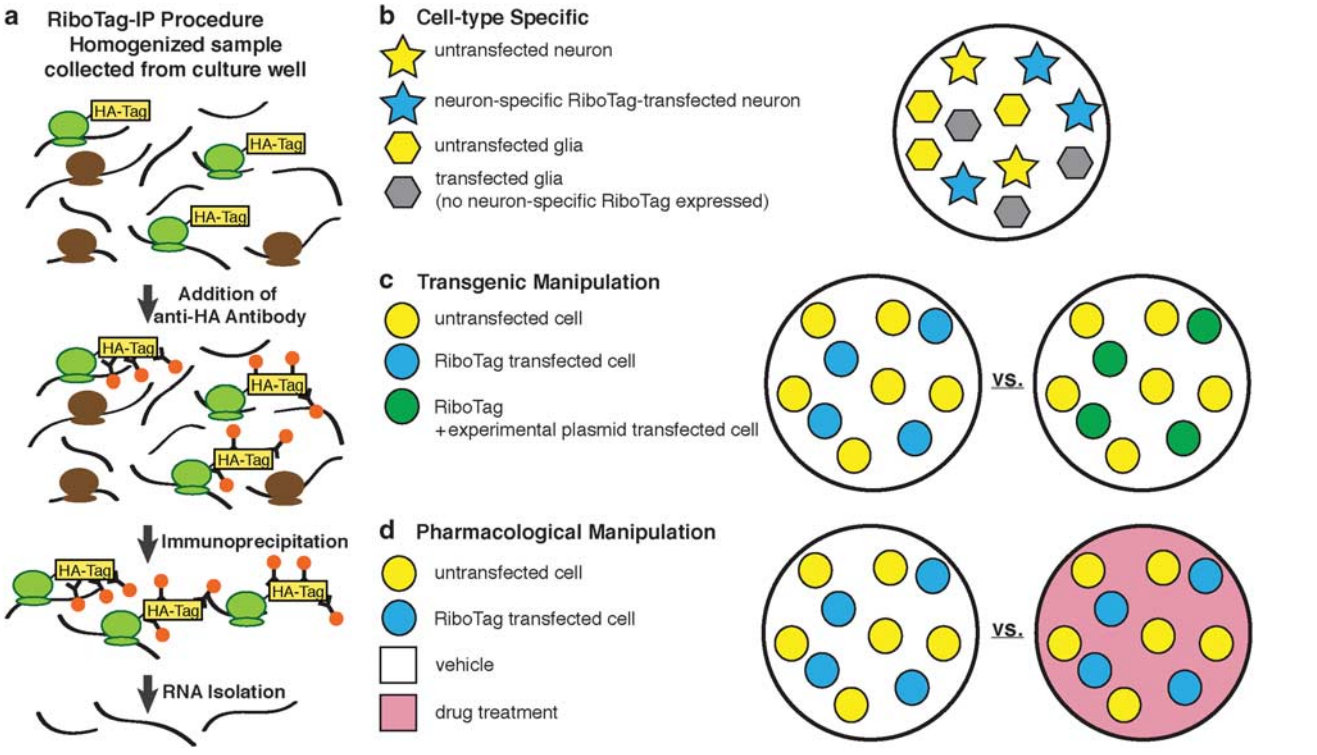

Figure 1. Examples of *in vitro* RiboTag applications for primary cultures. (a) Representation of translating RNA isolation using RiboTag immunoprecipitation. (b) Cell-type specific: cultures containing mixed cellular populations are transfected with RiboTag plasmids that restrict RiboTag expression to specific cells using cell type-specific promoters. RiboTag-isolated RNA from specific cell types can then be quantified. (c) Transgenic manipulation: cell cultures are transfected with RiboTag alone or co-transfected with transgenes that enhance or inhibit expression of specific proteins. RiboTag-isolated RNA can measure the effect of transgenic manipulations on RNA translation. (d) Pharmacological manipulation: cultures are transfected with RiboTag and then treated with drug or vehicle. Drug-induced changes in RNA translation can then be measured using RiboTag-isolated RNA from vehicle and drug-treated samples.

useful technique has only begun to be realized and will surely lead to novel insights into the complex regulation of gene expression.

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Pharmacological Treatments for Autism Spectrum Disorder: Will Emerging Approaches Yield New Treatments?

Advances in modern genetics are rapidly changing the way we approach autism spectrum disorder (ASD) and other complex brain disorders. For example, massive sequencing efforts have identified over 50 'high confidence' genes that possess intrinsic diagnostic and predictive value for ASD (De Rubeis *et al*, 2014; Iossifov *et al*, 2014). A *post hoc* analysis reveals that these genes encode protein products that are primarily localized to post-synaptic boutons and are involved in synthesis of synaptic proteins. Preclinical studies have begun to stratify syndromic forms of autism into groups defined by varying degrees of excitatory/inhibitory imbalance. Importantly, the phenotypic overlap among these disorders has provided optimism that viable therapeutics might emerge that show efficacy in both monogenetic and idiopathic ASD populations due to similarly disrupted signaling pathways.

Perhaps the most well studied potential therapeutic mechanism is that of metabotropic glutamate receptor 5 ($mGlu_5$) antagonism in fragile X syndrome (FXS), where genetic and pharmacological strategies of reducing $mGlu_5$-dependent protein synthesis have shown robust preclinical efficacy. However, the failure of two phase 2 clinical trials has caused many to question whether the target is viable (Jacquemont *et al*, 2014). An alternative approach is use of the $GABA_B$ receptor agonist arbaclofen, which normalizes excessive protein synthesis and excitatory/inhibitory imbalance in FXS model mice. While a phase 2b clinical trial failed to achieve its primary endpoint of treating irritability, *post hoc* analysis with the Aberrant Behavior Checklist -Social Avoidance scale, a recently validated scale for the assessment of FXS, showed a treatment effect in the full study population. A *post hoc* subgroup of 27 subjects with more severe social impairment also showed improvements on the Vineland II socialization raw scores and on the Aberrant Behavior Checklist-Social Avoidance scale (Jacquemont *et al*, 2014).

Another ASD treatment strategy that is gathering momentum is the targeting of pleiotropic growth factors. In the case of Rett syndrome, small molecules mimicking the effects of brain derived neurotrophic factor or insulin-like growth factor 1 (IGF1) have efficacy in respiratory, cognitive and survival measures in preclinical studies (Castro *et al*, 2014; Kron *et al*, 2014). In fact, a recent trial concluded that recombinant human IGF1 improved respiratory and behavioral parameters in Rett syndrome patients, and patients are currently being recruited for phase 2b trials (Khwaja *et al*, 2014). Likewise, the IGF1 synthetic peptide, NNZ-2566, normalized spine density, hyperactivity and synaptic protein synthesis in a mouse model of FXS, and patients are currently being enrolled for phase 1 clinical trials (Deacon *et al*, 2015).

One common thread among these next generation ASD treatment strategies is that they normalize excitatory/inhibitory balance, in part, through the modulation of protein synthesis-dependent synaptic plasticity. These novel targets represent new access points to a pathway of genes disrupted in ASD patients, which may provide greater translational value than $mGlu_5$ antagonism. In addition, the recent failure of $mGlu_5$ modulators in FXS clinical trials does not invalidate the target, but rather highlights a need for a more complete understanding of the temporal, spatial and mechanistic subtleties underlying the inability of preclinical studies to translate to clinical populations, and the need to carefully consider patient stratification and appropriate outcome measures. Although it is too early to predict the ultimate impact of these advances on treatment of ASD, a renewed emphasis on these finer points of therapeutic design, coupled with the emergence of exciting new targets, represents important progress toward effective ASD treatments.

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