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FOCUS ARTICLE

Uncovering cell type-specific complexities of gene expression and RNA metabolism by TU-tagging and EC-tagging

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Cell type-specific transcription is a key determinant of cell fate and function. An ongoing challenge in biology is to develop robust and stringent biochemical methods to explore gene expression with cell type specificity. This challenge has become even greater as researchers attempt to apply high-throughput RNA analysis methods under in vivo conditions. TU-tagging and EC-tagging are in vivo biosynthetic RNA tagging techniques that allow spatial and temporal specificity in RNA purification. Spatial specificity is achieved through targeted expression of pyrimidine salvage enzymes (uracil phosphoribosyltransferase and cytosine deaminase) and temporal specificity is achieved by controlling exposure to bioorthogonal substrates of these enzymes (4-thiouracil and 5-ethynylcytosine). Tagged RNAs can be purified from total RNA extracted from an animal or tissue and used in transcriptome profiling analyses. In addition to identifying cell type-specific mRNA profiles, these techniques are applicable to noncoding RNAs and can be used to measure RNA transcription and decay. Potential applications of TU-tagging and EC-tagging also include fluorescent RNA imaging and selective definition of RNA–protein interactions. TU-tagging and EC-tagging hold great promise for supporting research at the intersection of RNA biology and developmental biology.

This article is categorized under:
Technologies > Analysis of the Transcriptome

KEYWORDS
4-thiouracil, 5-ethynylcytosine, biosynthetic RNA tagging, cell type-specific gene expression, mRNA decay, transcription

1 | INTRODUCTION

Development requires precise deployment of genetic information in the form of messenger RNAs and noncoding RNAs. Biologists have begun uncovering the complexities of this process by combining two strategies: cell type-specific RNA purification and transcriptome profiling. Multiple methods for cell type-specific RNA purification are available and the method of choice influences the type of information obtained, as reviewed previously (Handley, Schauer, Ladurner, & Margulies, 2015). Physical isolation of cells (typically by fluorescence-activated cell sorting or laser-capture microdissection) is useful for obtaining all coding and noncoding RNAs but removing cells from their tissue microenvironment can induce changes in RNA expression (van den Brink et al., 2017). A popular alternative approach, tandem ribosome affinity purification (TRAP), uses targeted expression of an epitope-tagged ribosomal protein to immunoprecipitate ribosome-mRNA complexes. TRAP avoids the side effects caused by cell isolation but only interrogates the translating mRNA population. Biosynthetic RNA tagging is a cell type-specific RNA purification method that provides several advantages over commonly used alternatives. Biosynthetic RNA tagging uses transgenic expression of pyrimidine salvage enzymes to target incorporation of

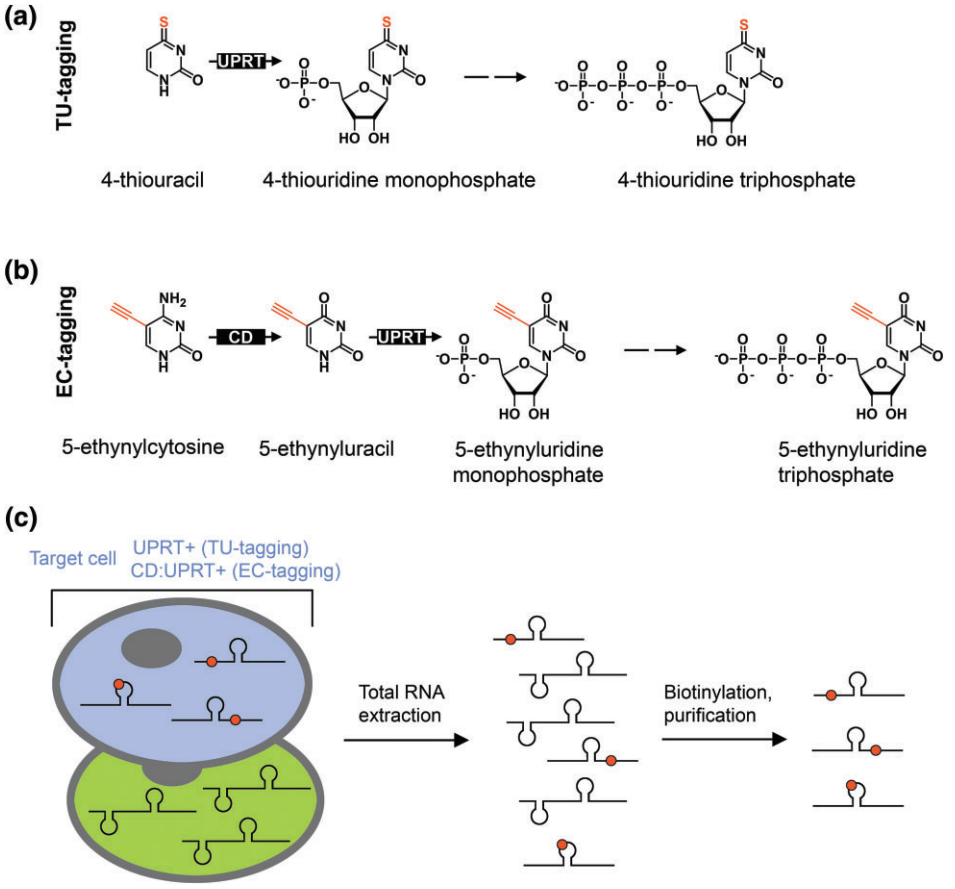

FIGURE 1 Biosynthetic RNA tagging pathways and basic RNA purification scheme. (a) Conversion of 4-thiouracil to 4-thiouridine monophosphate by uracil phosphoribosyltransferase (UPRT). The dashed arrow represents the phosphorylation of 4-thiouridine monophosphate by nucleotide kinases. (b) Conversion of 5-ethynylcytosine to 5-ethynyluracil by cytosine deaminase (CD) and conversion of 5-ethynyluracil to 5-ethynyluridine monophosphate by UPRT. The dashed arrow represents the phosphorylation of 5-ethynyluridine monophosphate by nucleotide kinases. (c) The bioorthogonal nucleotide produced in target cells (orange circle) is incorporated into nascent RNAs. Tagged RNAs are purified from a mixture of all RNAs by selective biotinylation and capture on streptavidin beads

bioorthogonal nitrogenous bases into newly transcribed RNA. Tagged RNA is then purified from intact animals or tissues. Biosynthetic tagging provides spatial specificity without physical isolation of cells, allowing physiologically relevant measurements of in vivo transcription. Biosynthetic tagging also provides temporal control that can be used to study RNA metabolism. This article summarizes strengths, limitations, and applications of two cell type-specific biosynthetic tagging methods currently used in model organisms: TU-tagging and EC-tagging.

2 | A BRIEF HISTORY OF TU-TAGGING

TU-tagging was originally developed as a way to obtain parasite-specific mRNAs from tissue culture cells infected with *Toxoplasma gondii* (Cleary, Meiering, Jan, Guymon, & Boothroyd, 2005). *T. gondii* uses the enzyme uracil phosphoribosyltransferase (UPRT) to convert uracil into uridine monophosphate that is ultimately incorporated into RNA while its mammalian host cells have little or no UPRT activity (Pfefferkorn & Pfefferkorn, 1977). Building upon work that identified UPRT-binding uracil analogs,(Iltzsch & Tankersley, 1994) 4-thiouracil (4TU) was shown to be a nontoxic substrate for *T. gondii* UPRT (Figure 1a). Only parasite RNA incorporated 4TU in infected cells and tagged RNAs were purified using thiol-specific biotinylation and streptavidin purification (Figure 1c) (Cleary et al., 2005). The ability to engineer cell type-specific RNA tagging was demonstrated by expressing *T. gondii UPRT* (*T.g.UPRT*) in HeLa cells (Cleary et al., 2005). These experiments laid the foundation for TU-tagging in model organisms, first achieved in *Drosophila* using the *Gal4-UAS* system to drive cell type-specific *T.g.UPRT* expression (Miller, Robinson, Cleary, & Doe, 2009). *Drosophila* TU-tagging has been used to study gene expression in multiple cell types, including embryonic neurons, larval brain neuroblasts, ovarian escort cells and gustatory receptor neurons (Burow et al., 2015; Inagaki et al., 2012; Liu et al., 2015; Syed, Mark, & Doe, 2017).

TU-tagging has also been established in mice and zebrafish. Mouse TU-tagging has identified mRNAs expressed in small populations of brain cells and measured transcription kinetics in spleen endothelial cells following lipopolysaccharide challenge (Chatzi, Zhang, Shen, Westbrook, & Goodman, 2016; Gay et al., 2013). Zebrafish TU-tagging has compared gene expression in differentiating neurons of the embryonic and adult nervous system and discovered novel sensory hair cell-specific transcripts (Erickson & Nicolson, 2015; Tallafuss et al., 2015). Some degree of nonspecific (transgenic UPRT-independent) RNA-tagging has been reported in all model organisms where TU-tagging has been applied. In *Drosophila*, nonspecific RNA tagging is caused by an endogenous UPRT enzyme (Ghosh, Shimell, Leof, Haley, & O'Connor, 2015) and this background usually

TABLE 1 Main differences between TU-tagging and EC-tagging

	TU-tagging	EC-tagging
Pyrimidine salvage enzyme	UPRT	CD and UPRT; expressed in combination or as a single CD:UPRT fusion
Bioorthogonal nucleobase	4-Thiouracil	5-Ethynylcytosine
Tag chemistry	Thiol-reactive biotin (HPDP-biotin or MTS-biotin); reversible by reduction	"Click chemistry" addition of biotin, fluorophores; not reversible
Specificity	May be limited by endogenous uracil salvage activity in metazoans	Lack of cytosine deaminase activity in metazoans enhances specificity

necessitates tissue dissection following 4TU exposure to improve signal to noise ratios (Miller et al., 2009). In addition to non-specific tagging via endogenous UPRT, uracil can be converted to uridine monophosphate by orotate phosphoribosyltransferase, the penultimate enzyme in de novo pyrimidine synthesis, or the sequential activity of uridine phosphorylase and uridine kinase, but these routes are much less efficient than the UPRT pathway (Carter, Donald, Roos, & Ullman, 1997).

3 | A BRIEF HISTORY OF EC-TAGGING

EC-tagging improves cell type specificity by requiring the activity of two enzymes: cytosine deaminase (CD) and UPRT. The sequential activity of CD and UPRT converts the bioorthogonal nucleobase 5-ethynylcytosine (5EC) into 5-ethynyluridine monophosphate (Figure 1b) (Hida et al., 2017). Conversion of cytosine to uracil has not been described in metazoans, so targeted expression of CD (derived from *Saccharomyces cerevisiae*) significantly increases cell type specificity. The ethynyl group in 5-ethynyluridine (5EUd) is advantageous for RNA tagging because it allows efficient coupling of modifiers (biotin, fluorophores) via copper (I)-catalyzed cycloaddition or "click chemistry." Prior to EC-tagging, RNA tagging with the nucleoside 5EUd was described in tissue culture cells (Jao & Salic, 2008). 5EUd is efficiently transported into animal cells and incorporated into nascent RNAs without requiring any genetic modification. 5EUd tagging has been used to measure nascent transcription, mRNA turnover, and localization of fluorescently tagged RNAs (Abe et al., 2012; Akbalik et al., 2017; Percharde, Wong, & Ramalho-Santos, 2017). EC-tagging supports the same types of analysis but has the advantage of cell type specificity. 5EC is not currently commercially available but synthesis is straightforward and uses standard organic chemistry methods (Hida et al., 2017). EC-tagging has been used in cultured mammalian cells and *Drosophila* and should be applicable to any system where transgene expression is possible. Expression of CD and UPRT (both genes derived from *S. cerevisiae*) may be achieved using distinct enhancers for each gene or through expression of a single CD:UPRT fusion. Table 1 summarizes the main differences between TU-tagging and EC-tagging.

4 | THINGS TO CONSIDER WHEN USING TU-TAGGING AND EC-TAGGING

4.1 | Reference sample

Cell type-specific biosynthetic RNA tagging experiments typically compare newly synthesized RNA to reference RNA collected from all cells. The source of reference RNA is an important parameter to consider. As previously described,(Gay, Karfilis, Miller, Doe, & Stankunas, 2014) the reference may be bulk input RNA (a type I experiment) or biosynthetically tagged and purified RNA (a type II experiment). Type I experiments work best for identifying RNAs whose transcription is strictly limited to target cells (Figure 2). This is because a type I reference lacks temporal specificity: all RNAs are present while TU-tagging/EC-tagging selects RNAs made during a defined time window. An mRNA that is transcribed in target and nontarget cells is therefore likely to be present at higher levels in the reference (assuming the RNA is of average stability) even if the gene is transcribed at a higher rate in target cells. In contrast, type II experiments allow identification of differences in transcription since the reference only contains tagged RNAs synthesized during the same time window used for TU-tagging/EC-tagging (Figure 2). Type II reference RNA may be prepared using ubiquitous tagging via UPRT/CD:UPRT or exposure to the relevant nucleosides: 4-thiouridine (4TUd) for TU-tagging and 5EUd for EC-tagging (Hida et al., 2017). These nucleosides are taken up by all cells and enter RNA biosynthetic pathways independent of UPRT or CD:UPRT. Another advantage of the type II design is that reference RNA is subject to the same purification steps as the TU-tagged/EC-tagged RNA, limiting the chance that a technical bias may be introduced into only one sample (Tomorsky, DeBlander, Kentros, Doe, & Niell, 2017).

4.2 | Cellular responses to RNA tagging

Experimentally induced changes in transcription or cell physiology are a concern in any cell type-specific RNA purification technique. Ubiquitous expression of UPRT or CD:UPRT has no effect on *Drosophila* development and feeding wildtype

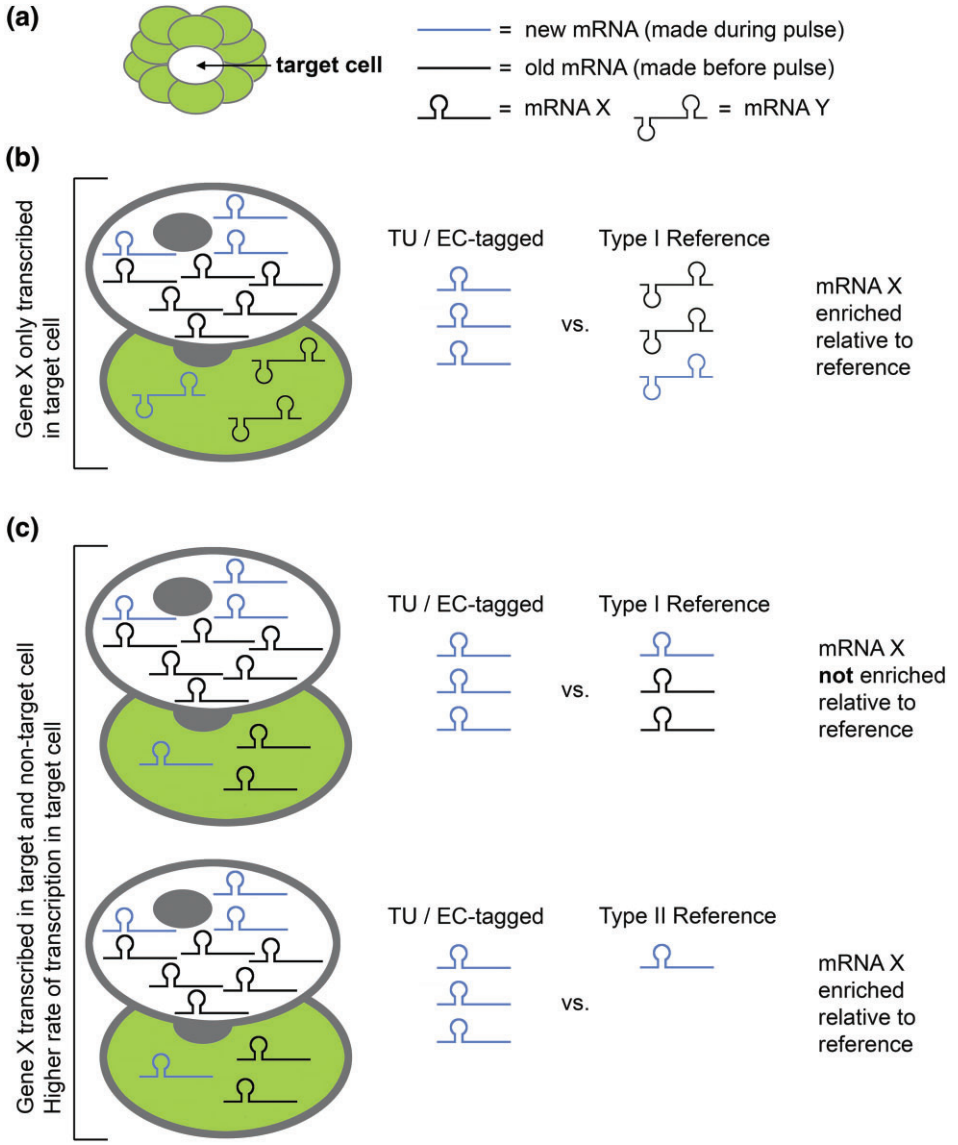

FIGURE 2 Reference RNA type affects TU-tagging and EC-tagging transcriptome profiling results. (a) Key: the target cell expressing UPRT or CD:UPRT is white, nontarget cells are green. Biosynthetically tagged mRNA is blue, nontagged mRNA is black. Distinct mRNAs (X and Y) are represented by different shapes. (b) When gene X is only transcribed in the target cell, a type I reference (containing tagged and nontagged mRNAs) is sufficient to reveal that mRNA X is target cell-specific. (c) When gene X is transcribed in both cell types, a type I reference may fail to reveal elevated expression of gene X in the target cell. In contrast, use of a type II reference containing only newly made mRNAs reveals that gene X is transcribed at higher levels in the target cell

larvae 4TU or 5EC similarly has no effect (Hida et al., 2017). Absence of gross developmental defects, however, does not rule out the possibility that certain tissues may respond to the introduction of extracellular pyrimidines. For example, uracil secreted by pathogenic bacteria activates the Hedgehog pathway in *Drosophila* gut epithelial cells and leads to production of reactive oxygen species (Lee et al., 2015). This pathway may be unique to the gut epithelium or only triggered by unmodified uracil since TU-tagging experiments have not produced evidence of antibacterial or other stress responses. The effects of RNA tagging on organismal development have been tested in *Drosophila* by continuously feeding 4TU/5EC to newly hatched larvae expressing UPRT/CD:UPRT in all cells. TU-tagging causes a minor developmental delay and EC-tagging causes a gradual decline in larval viability after 24 hr followed by growth arrest at the third instar stage (Hida et al., 2017). The mechanism underlying these developmental defects is not known but high doses of 4TUd inhibit ribosomal RNA synthesis in mammalian tissue culture cells(Burger et al., 2013) and similar inhibition may occur following prolonged RNA tagging in *Drosophila.* Importantly, transcriptome profiling of mammalian cells and *Drosophila* subject to TU-tagging or EC-tagging for as long as 24 hr found no significant alteration of gene expression (Cleary et al., 2005; Hida et al., 2017). TU-tagging and EC-tagging experiments typically use exposure periods of 24 hr or less, so conditions that might alter gene expression or physiology are avoided as part of the standard experimental design. However, pilot studies aimed at identifying side effects may be warranted depending on the experiment.

4.3 | Purification and quantitation of tagged RNAs

RNA purification in TU-tagging and EC-tagging experiments begins with biotinylation of modified uridines. 4TUd is biotinylated via disulfide bond formation between the C4 sulfur and a thiol-specific biotinylation reagent. Early TU-tagging experiments used HPDP-biotin but MTS-biotin was recently shown to be a much more efficient biotinylation reagent (Duffy et al.,

2015). This improved biotinylation chemistry is expected to increase yields in animal TU-tagging experiments (although nonspecific TU-RNA yields will also increase). MTS-biotin should also eliminate the uridine content bias that was originally described in TU-tagging experiments (Miller et al., 2009). If the biotinylation reaction is highly efficient, a transcript with a single tagged uridine should be purified as efficiently as a transcript with multiple tagged uridines. This appears to be the case for EC-tagging, where no uridine content bias was detected (Hida et al., 2017). Since the click chemistry used to biotinylate 5-ethylyuridine is nonreversible, analysis of RNAs purified by EC-tagging usually requires cDNA synthesis while the tagged RNA is bound to streptavidin beads. The cDNA can then be eluted using heat or RNAse H treatment. In contrast, the disulfide bond linking biotin to 4TUd is reversible and allows elution of TU-tagged RNA from streptavidin beads. During cDNA synthesis, reverse transcriptase (RT) reads through 4TU in purified TU-tagged RNAs(Cleary et al., 2005) but terminates upon encountering biotinylated 5EU. RT termination does not appear to bias EC-tagging experiments (at least those that use a type II reference design) and priming with random oligonucleotides should allow cDNA synthesis across the length of purified transcripts. For TU-tagging, it may be possible to skip the purification of biotinylated TU-tagged RNAs and directly quantify tagged RNAs by sequencing. Certain chemical modifications cause RT to misread 4TUd as cytosine and this mutation can be quantified by RNA sequencing (Herzog et al., 2017; Riml et al., 2017). Application of this direct sequencing approach to TU-tagging in animals will require deep sequencing to effectively quantify modified transcripts.

Messenger RNA and ribosomal RNA labeling has been demonstrated for TU-tagging and EC-tagging, but these methods have yet to be applied to the analysis of other RNA species. Bias toward purification of any specific type of RNA is not expected (assuming the bioorthogonal nucleotides are equally efficient substrates for all RNA polymerases), but purification of small RNAs (tRNAs, microRNAs) may be hindered by low frequency of modified uridine incorporation. Incorporation frequency in total RNA has been calculated for *T. gondii* exposed to 4TU for 6 hr (4TUd was incorporated at a frequency of 1:26 uridines)(Cleary et al., 2005) and CD:UPRT-positive HeLa cells exposed to 5EC for 6 hr (5EUd was incorporated at a frequency of 1:77 uridines [R. Spitale and M. Cleary, unpublished results]). These frequencies can be used to calculate the likelihood that a RNA molecule of a given length will incorporate a tagged uridine (based on the Poisson distribution). For 22 nucleotide microRNA with equal representation of all four nucleotides, 19% are expected to contain at least one tag by TU-tagging and 7% are expected to contain at least one tag by EC-tagging. While yields of small RNAs are expected to be low, coupling TU-tagging or EC-tagging with RNA-sequencing protocols designed to enrich small RNAs(Hafner et al., 2012) could enable identification of cell type-specific tRNAs or microRNAs.

4.4 | Sensitivity and cell type specificity

The ability of TU-tagging and EC-tagging to identify mRNAs from rare cell types is well documented. An early test of mouse TU-tagging aimed to identify transcripts from endothelial cells and microglia in the adult brain (Gay et al., 2013). These cells make up about 5% of the brain and TU-tagging enriched known endothelial and microglial transcripts, depleted neural-specific transcripts, and identified novel endothelial-specific mRNAs. The sensitivity and specificity of EC-tagging was tested by transcriptome profiling mushroom body neurons in the *Drosophila* larval brain (Hida et al., 2017). EC-tagging effectively purified mushroom body-specific mRNAs from total larval RNA, even though mushroom body neurons only comprise about 0.07% of larval cells. The ability of TU-tagging to purify mushroom body mRNAs from total larval RNA was tested in matching experiments (Hida et al., 2017). As expected, TU-tagging failed to enrich mushroom body-specific transcripts due to nonspecific RNA tagging throughout larvae. TU-tagging performs best when tissue containing the target cells is dissected following 4TU exposure and mushroom body mRNA purification would likely have been successful if dissected larval central nervous system was used as input RNA, as demonstrated for TU-tagging targeting larval brain glia and neuroblasts (Syed et al., 2017). One option for improving TU-tagging specificity is fragmentation of input RNA prior to biotinylation and purification. RNA from nontarget cells has a low frequency of 4TUd incorporation compared to RNA from transgenic UPRT-positive cells, so fragmentation decreases the likelihood of purifying nonspecific RNA (Gay et al., 2013). Another option for improving TU-tagging is to engineer transgenic UPRT expression in flies that lack the endogenous UPRT gene *krishah* (*kri*). Growth defects in *kri* loss-of-function mutants are reversed by culturing larvae in media supplemented with uracil or uridine(Ghosh et al., 2015) and this could be combined with conditional 4TU feeding to direct TU-tagging in a *kri* mutant background, although this approach has not been tested. A potential source of nonspecific RNA tagging in TU-tagging and EC-tagging is excretion of modified uridine. 4TUd and 5EUd produced in UPRT/CD:UPRT-positive cells might be excreted and taken up by neighboring nontarget cells. This was demonstrated by treating CD:UPRT-negative cells with conditioned media from CD:UPRT-positive cells exposed to 5EC (Hida et al., 2017). The conditioned media caused weak RNA tagging in the negative control cells, apparently due to excreted 5EUd (or possibly 5-ethynyluracil). Transfer of tagged uridines may be unique to tissue culture experiments since there is no evidence to suggest significant nucleoside excretion in animal experiments, although this is difficult to absolutely rule out.

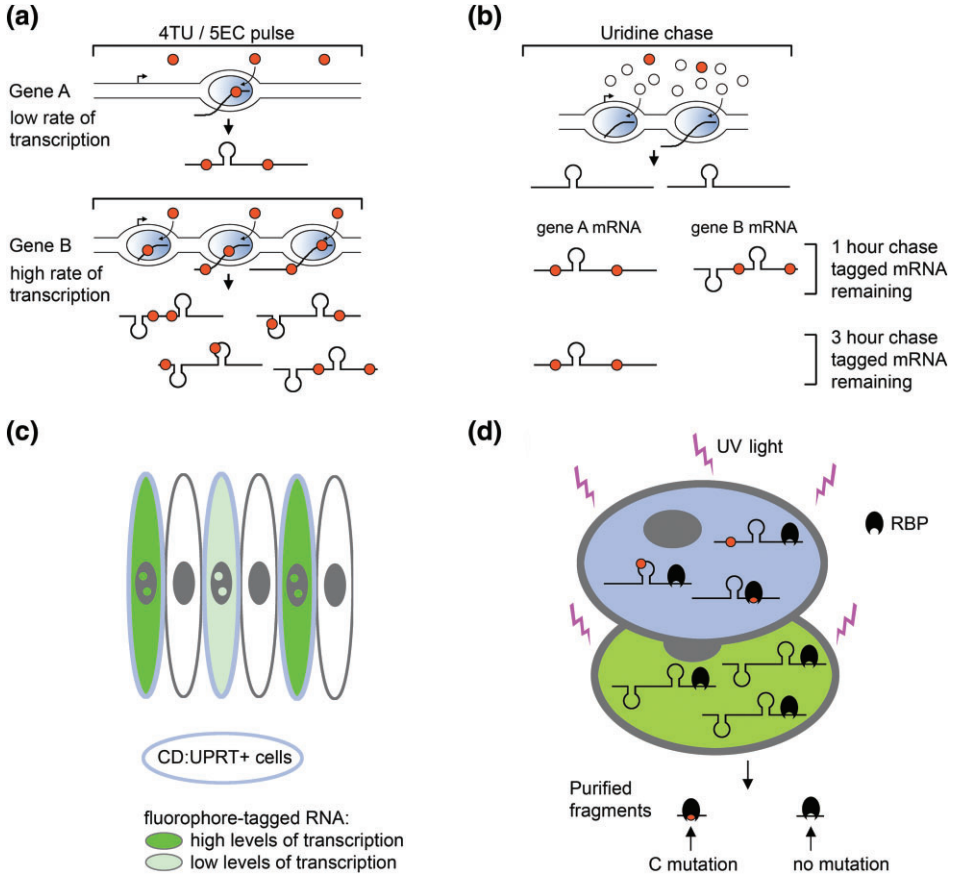

FIGURE 3 Applications beyond standard transcriptome profiling. (a) Relative transcription activity can be measured using 4-thiouracil (4TU) or 5-ethynylcytosine (5EC) pulse tagging. In this example, a single gene A transcript is made during the pulse compared to four gene B transcripts, indicating a higher rate of transcription at gene B. (b) RNA decay can be measured by following a 4TU or 5EC pulse with a uridine chase. Excess uridine ensures no new tagged RNAs are made during the chase and tagged RNAs are made during the pulse can be quantified during a chase timecourse. In this example, gene A mRNA is more stable (all gene B mRNAs are degraded after 3 hr). (c) Cell type-specific RNA metabolism can be analyzed by visualizing ethynyluridine-tagged RNAs. Fluorophore-coupling by "click chemistry" in fixed tissue samples can be restricted to specific cell types (the CD:UPRT-positive target cells). In this example, the middle CD:UPRT-positive cell shows a general decrease in RNA synthesis compared to other target cells, including decreased ribosomal RNA synthesis revealed by weak nucleolar fluorescence (green circles in the nucleus). (d) Cell type-specific RNA-binding protein (RBP) targets can be identified by TU-tagging combined with PAR-CLIP. UV light enhances crosslinking of the RBP to 4-thiouridines in target cells. Following immunoprecipitation and sequencing of RBP-associated RNA fragments, only the sequences derived from target cells will have the characteristic thymidine to cytosine transition

5 | APPLICATIONS BEYOND TRANSCRIPTOME PROFILING

5.1 | Measure RNA synthesis

Newly transcribed RNA capture can be used to measure transcription rates or compare relative levels of RNA synthesis (Figure 3a). Brief pulses of 4TUd have been used to directly measure transcription rates in yeast and mammalian cells (Dölken et al., 2008; Miller et al., 2011). Precise measurement of transcription rates using TU-tagging or EC-tagging in animals is complicated by the influence of organismal metabolism on 4TU/5EC delivery to target cells. Brief pulse labeling by TU-tagging or EC-tagging in animals also results in very low yields of tagged RNA. The limitation of low yields could be overcome by physical enrichment of relevant tissue following the 4TU/5EC pulse combined with RNA-sequencing methods optimized for low input RNA (Farris, Wang, Ward, & Dudek, 2017). Even 4TU exposures spanning several hours can provide enrichment of newly transcribed RNAs, as demonstrated by mouse TU-tagging experiments. For example, intron sequences were highly abundant in RNA-seq libraries made from brain endothelial cells and microglia following a 4–6-hr 4TU exposure, indicating selective capture of recently transcribed unspliced mRNAs (Gay et al., 2013). TU-tagging in immature dentate granule neurons also provided evidence of nascent transcript enrichment (Chatzi et al., 2016). When compared to RNA obtained by fluorescence-activated cell sorting of the same immature neurons, only the TU-tagging dataset contained synapse-related mRNAs. This revealed that transcription of synaptic genes begins well before neuron maturation and synaptogenesis. This result also demonstrated how total RNA measurements may not be ideal for studying the timing of gene expression.

5.2 | Measure RNA decay

RNA abundance is determined by synthesis and decay rates and this relationship can be used to calculate RNA half-lives based on transcription and abundance measurements. This approach has proven useful in yeast and mammalian cells but requires steady state RNA metabolism conditions (Miller et al., 2011; Russo, Heck, Wilusz, & Wilusz, 2017). Cells undergoing developmental transitions are unlikely to meet this steady state requirement. Instead, cell type-specific RNA decay can be measured using a pulse-chase approach (Figure 3b). This approach avoids the use of chemical inhibitors of RNA polymerases or conditional RNA polymerase mutants, traditional means of measuring mRNA decay that can cause confounding side effects. Pulse-chase TU-tagging has been used to obtain mRNA decay measurements across all tissues and specifically in the nervous system of *Drosophila* embryos (Burow et al., 2015). These studies discovered neural-specific decay kinetics, including stabilization of transcripts encoding regulators of axonogenesis and destabilization of transcripts encoding ribosomal proteins. Neural-specific mRNA decay analysis also identified a role for the RNA binding protein (RBP) Pumilio in controlling the stability of neural fate-associated mRNAs.

5.3 | Image-tagged RNA

Coupling fluorophores to 5EUd in biosynthetically tagged RNAs allows visualization of bulk RNA turnover and assessment of relative levels of global transcription in different tissues (Jao & Salic, 2008). Fluorophore addition is performed after in vivo RNA tagging using fixed and permeabilized cells or tissues. This approach was recently used to image-tagged RNA in cultured hippocampal neurons and larval zebrafish brains (Akbalik et al., 2017). Imaging across a timecourse of 5EUd exposure revealed the trafficking kinetics of RNA granules within axons and activity-dependent increase in RNA synthesis within specific brain regions. EC-tagging could be used to perform similar experiments in vivo, with RNA imaging targeted to specific cell types (Figure 3c).

5.4 | Identify RNA–protein interactions

4TUd enables highly specific mapping of protein binding sites using PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) (Hafner et al., 2010). PAR-CLIP takes advantage of two properties of 4TUd: 4TUd excited with UV light (>310 nm) forms photoadducts with associated proteins and 4TUd at crosslinked sites is misread as cytosine during reverse transcription. Following crosslinking, cell lysis, and RNAse digestion, an RBP of interest can be immunoprecipitated along with the protected RNA fragment. Sequencing purified RNAs identifies RBP binding sites and the absence of a uridine to cytosine transition allows elimination of contaminating nonspecific reads. 4TUd crosslinking may also be used for unbiased discovery of RBPs. TU-tagging has been used in this context to identify proteins bound to maternally deposited mRNAs in *Drosophila* embryos (Wessels et al., 2016). For this study, UPRT was expressed in nurse cells of the female germline (where maternal mRNAs are transcribed) and 4TU was delivered by feeding adult flies. TU-tagging followed by 4TUd crosslinking significantly increased the yield and diversity of proteins obtained by poly(A) + RNA selection. One caveat of combining PAR-CLIP and TU-tagging is that low 4TUd frequency could limit detection of proteins bound at specific sites, but the success of TU-tagging in defining the maternal mRNA interactome suggests that cell type-specific PAR-CLIP may be achieved using the proper parameters and sensitive sequencing methods.

6 | CONCLUSIONS AND FUTURE DIRECTIONS

Biosynthetic tagging is a useful option for obtaining cell type-specific RNAs, particularly when physical isolation of target cells is technically unfeasible or when unbiased measurements of RNA metabolism are desired. TU-tagging and EC-tagging have primarily been used to quantify cell type-specific mRNA levels, but these techniques can also be used to study RNA transcription, RNA decay, and other events during the lifetime of a RNA. Biosynthetic RNA tagging technology will likely continue evolving and improving. Bioorthogonal nucleotide precursors with RNA polymerase selectivity or different chemical reactivities could expand the types of information obtained by biosynthetic RNA tagging (Nainar et al., 2016). Promising alternative means of achieving cell type specificity include targeted expression of penicillin G amidase (this enzyme "uncages" a 2′-azidoadenosine nucleoside that is subsequently added to poly(A) tails via cytoplasmic poly(A) polymerase) and photoregulated uncaging of 5-ethynyluracil (Feng, Li, & Spitale, 2017; Nguyen et al., 2017). Additional specificity may be achieved through reconstitution of functional CD from independently expressed fragments. This "split-CD" approach has been demonstrated for CD fragments fused to complementary leucine zipper domains(Hida et al., 2017) and could potentially be used to engineer small molecule-dependent or light-induced CD activation for EC-tagging. Creative application of existing methods and novel technical variations are sure to expand the use of cell type-specific biosynthetic RNA tagging to answer important questions in developmental biology.

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CONFLICT OF INTEREST

The author has applied for a patent covering EC-tagging technology.

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