single cells which include but are not preferential for CTCF sites (Bintu et al., 2018). Such complementary approaches for the investigation of regulatory architecture will be important avenues for future understanding.

The emerging view is that pairwise interactions do not capture the full complexity of chromatin structure, especially when considering population-based contact methods. Inevitably, future goals will involve maximizing resolution of interacting elements without sacrificing the number of interacting partners that can be detected, and Tri-C takes important steps toward this aim. Characterization of complex multi-way *cis*-regulatory architecture will dramatically contribute to our understanding of precise and robust gene expression control.

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3′ UTRs in the Eye of the TIGER

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Many human genes generate mRNAs with alternative 3′ untranslated regions (3′ UTRs), which can modulate protein function. In a recent *Cell* paper, Ma and Mayr (2018) unveil a major membraneless organelle, termed the TIS granule-endoplasmic reticulum (TIGER), which enables specific 3′ UTRs to encipher protein localization.

Membrane-delimited organelles sort and compartmentalize biochemical reactions within eukaryotic cells. More recently, membraneless organelles (MLOs) have come to prominence as cellular structures that arise via liquid-liquid phase separation (LLPS), resulting in a switch-like condensation of biomolecules into concentrated liquid and gel-like assemblies (Banani et al., 2017; Gomes and Shorter, 2018). A growing list of such condensates has been identified, but the extent to which they serve as bioreactors in a manner analogous to classical organelles or serve as passive storehouses to buffer protein and RNA concentrations remains unclear (Banani et al., 2017; Gomes and Shorter, 2018). In a recent paper in *Cell*, Ma and Mayr (2018) discover a major MLO and in the process provide insight not only into the function of MLOs, but also into the mechanism by which 3′ untranslated regions (3′ UTRs) transmit genetic information to proteins encoded by the corresponding mRNA (Berkovits and Mayr, 2015; Mayr, 2018).

In the paper, using elegant molecular cell biology, Ma and Mayr delineate a mechanism for information transfer from 3′ UTR to protein involving an assembly that adds to the list functional MLOs (Ma and Mayr, 2018). This MLO is highly enriched in the RNA-binding protein (RBP) TIS11B and is closely associated with the endoplasmic reticulum (ER). The authors have therefore dubbed it the TIGER (TIs granule-ER) domain (Figure 1) (Ma and Mayr, 2018). Their work suggests that TIGER domains may uniquely compartmentalize the translation and specific 3′ UTR-mediated protein-protein interactions of certain membrane proteins, such as CD47 and PD-L1, provided their mRNAs contain a long 3′ UTR harboring multiple AU-rich elements (AREs) (Ma and Mayr, 2018).

The CD47 mRNA has two isoforms: one with a long and one with a short 3′ UTR. The long 3′ UTR isoform engages the RBP HuR, which recruits another protein, SET, which in turn binds newly translated CD47 protein (Figure 1). SET binding to CD47 promotes plasma membrane localization, where CD47 functions to prevent self-recognition by the immune system (Berkovits and Mayr, 2015; Ma and Mayr, 2018). By contrast, the CD47 protein that is translated from the short 3′ UTR isoform does not engage SET and is localized primarily to the ER (Berkovits and Mayr, 2015). However, the mechanism by which SET is transferred from CD47 mRNA to CD47 protein via HuR was not understood. Ma and Mayr (2018) now discover that TIS11B engages AREs, scaffolding the gel-like TIGER domain, which results in a unique microenvironment that facilitates the transfer of SET from target mRNA to translated protein (Figure 1) (Ma and Mayr, 2018). Thus, TIGERs provide a mechanism of solid-state information transfer from mRNA to protein by enabling proteins to remember information that is exclusively encoded in the 3′ UTR of their mRNA blueprint (Berkovits and Mayr, 2015; Ma and Mayr, 2018).

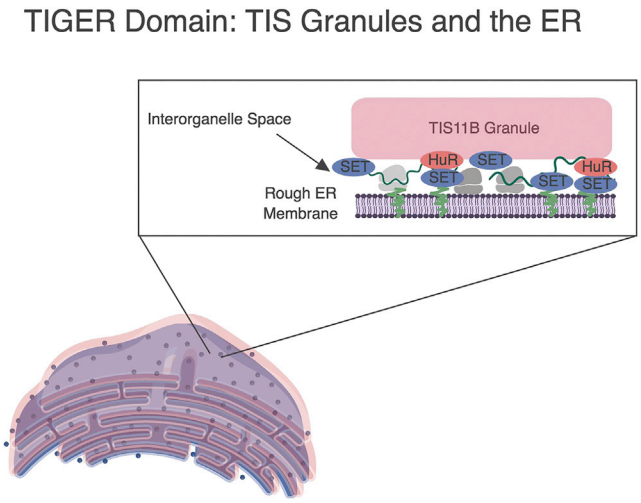

Figure 1. The TIGER Domain

The membraneless TIS11B granule (pink) covers portions of the cytoplasmic face of the rough endoplasmic reticulum (ER) (purple). Inset: the TIS11B granule is separated from the ER by an interorganelle space enriched for the protein SET. The microenvironment of the TIGER domain facilitates transfer of SET from mRNAs (dark green) to their encoded proteins (light green), ultimately destined for the plasma membrane. SET transfer is mediated in part by the protein HuR, which binds to specific AU-rich 3′ UTRs. Thus, TIS11B creates a specific environment, and HuR provides specificity, for 3′ UTR-mediated protein-protein interactions.

In a subsequent structure-function approach, Ma and Mayr dissect the sequence requirements for TIS11B granule assembly. They find that a distribution of opposing charges on the N- and C-terminal regions of TIS11B is required for TIS11B granule assembly (Ma and Mayr, 2018). Notably, this charge pattern within TIS11B is conserved and is reminiscent of other proteins that phase separate through interactions of opposing charge (Gomes and Shorter, 2018).

TIGERs exhibit many unique features. First, unlike many MLOs posited to be liquids that undergo rapid internal rearrangement and exchange of components with the bulk solution on a timescale of seconds, TIGERs are likely more gel-like, exhibiting FRAP recovery times for TIS11B on the order of minutes (Ma and Mayr, 2018). Liquid-like condensates typically relax into spheres (Banani et al., 2017). In contrast, TIGERs form a seemingly interconnected meshwork that encompasses, and moves with, portions of the peri-nuclear ER, resulting in an extensive MLO:classical organelle interface (Figure 1) (Ma and Mayr, 2018). Importantly, TIGERs enrich specific components while excluding others (Ma and Mayr, 2018). In particular, TIGERs concentrate membrane-protein-encoding mRNAs that harbor multiple AREs in their 3′ UTRs, as well as the cytoplasmic Hsp70 chaperone, HSPA8 (Ma and Mayr, 2018). This enrichment may foster a "folding organelle," which chaperones proteins prior to trafficking to the plasma membrane (Ma and Mayr, 2018). The intervening space between the MLO:classical organelle interface is enriched for SET, which itself is depleted from the TIS11B granule component of the TIGER (Figure 1) (Ma and Mayr, 2018). Ma and Mayr propose that translation of mRNAs with ARE-rich 3′ UTRs, within the specific microenvironment of the TIGER domain created by TIS11B, facilitates transfer of SET from mRNA to membrane proteins like CD47, whose folding may in turn be facilitated by localized enrichment of HSPA8 (Ma and Mayr, 2018).

TIGER domains enable a multi-layered, inter-organelle collaboration that functions to decode genetic information contained in a specific subclass of mRNA, including information encoded in the 3′ UTR, which would be lost in the translated polypeptide. This information ultimately informs protein localization across the cell, from the TIGER to the plasma membrane (Berkovits and Mayr, 2015; Ma and Mayr, 2018). These results thus address existing questions about 3′ UTR-mediated protein localization, but they also raise new questions about the formation of TIGERs and the potentially unique microenvironment that results. Numerous RBPs undergo LLPS, initially forming liquid droplets that can transition to less dynamic gels and aggregates (Gomes and Shorter, 2018). Does the enrichment of HSPA8 in TIGERs serve to chaperone nascent membrane proteins or to chaperone the TIS11B granule itself, preventing disadvantageous solidification? FRAP studies suggest the TIS11B granule is gel-like, and proteins in the TIGER appear less dynamic than their cytoplasmic counterparts (Ma and Mayr, 2018). Does the gelatinous matrix of the TIS11B granule serve to entrap SET in the interorganelle space, creating a locally high concentration favoring SET transfer to membrane proteins? Future studies with TIS11B mutants that fluidize or solidify the TIS granule may elucidate whether or not the material state of the TIS11B granule impacts SET localization within the TIGER and, ultimately, membrane protein localization. Alternatively, and not mutually exclusively, might a unique solvent environment within one or more layers of the TIGER alter the structure of RNAs, proteins, or both in a way that maximizes SET transfer to membrane proteins? Distinct solvent environments within condensates have been observed to significantly impact

nucleic acid structures (Nott et al., 2016) and might also impact TIGER functionality.

What other functions might TIGERs perform? Intriguingly, in yeast, overexpression of TIS11 buffers against the toxicity of TDP-43 and FUS (Kim et al., 2014; Sun et al., 2011), two RBPs heavily implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (Harrison and Shorter, 2017). It will be interesting to determine whether this ability to suppress toxicity of ALS/FTD-linked RBPs extends to TIS11B in human cells and whether TIGERs play a role.

The groundbreaking studies by Ma and Mayr have defined a unique MLO, the TIGER domain, endowed with the ability to facilitate the transduction of genetic information encoded in UTRs to mature proteins (Ma and Mayr, 2018). Positioned at the surface of a classical membrane-enveloped organelle, the TIGER represents a gelatinous boundary between the ER membrane and cytosol (Ma and Mayr, 2018). How the material state of the TIS11B granule enables functionality, the nature of the solvent environments the TIGER creates, and the biophysics governing its biogenesis remain intriguing questions for future studies.

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