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Multiplexed RNA trafficking in oligodendrocytes and neurons

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Summary

In oligodendrocytes and neurons genetic information is transmitted from nucleus to dendrites in the form of RNA granules. Here we describe how transport of multiple different RNA molecules in individual granules is analogous to the process of multiplexing in telecommunications. In both cases multiple messages are combined into a composite signal for transmission on a single carrier. Multiplexing provides a mechanism to coordinate local expression of ensembles of genes in myelin in oligodendrocytes and at synapses in neurons.

Keywords

HnRNP A2; myelin; RNA granules; synapse; TOG

Introduction

In neural cells genetic information, in the form of RNA granules, is transmitted from the nucleus to specialized subcellular compartments in the periphery where local translation occurs. In oligodendrocytes granules contain RNAs encoding proteins required for myelination of axons. In neurons granules contain RNAs encoding proteins required for synaptic contacts with axons. Although there are significant differences between oligodendrocytes and neurons in the molecular and cellular properties of the dendritic processes and in the morphology and function of the myelin compartment and dendritic spines, respectively (Figure 1), in both cell types the process of transmission of RNA molecules from nucleus to periphery is analogous to the process of multiplexing in telecommunications.

In multiplexing, different messages are combined into a composite signal by a device called a multiplexer (mux). The composite signal is transmitted over a single carrier to a network of receivers. Each receiver contains a device called a demultiplexer (dmux) that decomposes the composite signal into individual messages. One familiar example of multiplexing is cable television where multiple different television channels are combined into a composite signal that is transmitted over a single cable to a network of subscribers. Each subscriber has a receiver

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(TV) that uses a demultiplexer to filter out and display individual channels. Multiplexing minimizes transmission costs by using a single carrier to transmit an ensemble of different messages to multiple receivers.

In oligodendrocytes and neurons multiplexed transmission of different RNA molecules from nucleus to dendrites is mediated by similar molecular interactions (Figure 2). Selection of specific RNAs for multiplexing is mediated by sequence specific binding of the *trans*-acting protein heterogeneous nuclear ribonucleoprotein (hnRNP) A2 to *cis*-acting hnRNP A2 response elements (A2REs) in different RNAs. Linkage of multiple different A2RE RNAs together in the same granules is mediated by the multivalent scaffolding protein tumor overexpressed gene (TOG), which binds to multiple hnRNP A2 molecules. During transport of granules along microtubules, translation is suppressed by the translational inhibitor protein hnRNP E1. When granules become localized at sites of myelin formation in oligodendrocytes or in dendritic spines in neurons, individual multiplexed RNAs are translated into proteins required for myelination or synaptic plasticity, respectively. This review discusses molecular interactions that mediate selection, linkage, suppression and translation during multiplexed trafficking of RNAs in oligodendrocytes and neurons.

RNA localization

Historically RNA localization has been considered to be a specialized mechanism to achieve localized gene expression in cells with unusually polarized morphologies. However a recent high resolution *in situ* hybridization analysis of RNA localization during early *Drosophila* development revealed that > 70% of all transcripts exhibit characteristic intracellular localization patterns suggesting that most RNAs are localized and that RNA localization is an important mechanism for organizing many aspects of cellular architecture and function [1]. Furthermore, since many different transcripts show similar localization patterns, multiplexing may be a widespread mechanism for coordinating expression of ensembles of different genes in particular subcellular compartments.

In oligodendrocytes, myelin basic protein (MBP) RNA was first shown to be localized to the myelin compartment by subcellular fractionation [2] and subsequently by *in situ* hybridization [3,4]. Subcellular fractionation has since been used to identify additional myelin-enriched RNAs including: myelin oligodendrocytic basic protein, peptidyl arginine deiminase, ferritin heavy chain, eEF α 1, eEF δ 1, ribosomal proteins L7a and L21, SH3p13, KIF1A, dynein light intermediate chain mRNAs and others [5,6,7]. Tau and carbonic anhydrase II RNAs have also been identified in oligodendrocyte processes by *in situ* hybridization [4,8].

In neurons, many different mRNAs encoding post-synaptic proteins involved in synaptic plasticity are targeted to dendrites and translated locally in dendritic spines. A large scale *in situ* hybridization screen identified ~ 60 dendritically-localized RNAs in adult mouse brain [9]. Amplification and expression profiling techniques have identified several hundred additional dendritically localized RNAs in neurons [10].

Recent evidence indicates that several different RNAs that are localized to the myelin compartment in oligodendrocytes or to dendritic spines in neurons have common *cis/trans* trafficking determinants (A2RE and hnRNP A2, respectively) and are co-assembled into the same RNA granules and targeted to dendrites by the A2 pathway. Although this review is focused on multiplexed dendritic targeting of A2RE RNAs by the A2 pathway, there are examples of nonA2RE RNAs such as actin RNA that are targeted to dendrites by nonA2 pathways mediated by non-hnRNP A2 *trans*-acting factors (such as zipcode binding protein or staufen). It is possible that multiplexing is used in several different RNA localization pathways and may turn out to be a general mechanism for dendritic targeting of ensembles of RNAs with common *cis/trans* trafficking determinants.

RNA granules

RNA granules are supramolecular complexes designed for multiplexed transmission of genetic information from the nucleus to the myelin compartment in oligodendrocytes or to dendritic spines in neurons. RNA granules are characterized by existential instantiation, which means they are non-uniform in size, composition and structure. This distinguishes them from macromolecular assemblies such as ribosomes, proteosomes or nuclear pore complexes, characterized by universal instantiation meaning they are uniform in size, composition and structure. Non-uniformity of size, composition and structure makes RNA granules difficult to isolate and study by conventional biochemical or structural techniques. Recent attempts to isolate RNA granules by subcellular fractionation techniques and characterize them by microarray and proteomic techniques [14,15] have generated long and differing catalogues of RNA and protein components, including molecules known to be absent from RNA granules and lacking molecules that are known to be present in granules. Attempts to purify RNA granules may result in dissociation of authentic granule components and adventitious association of non-granule components during isolation, so that “isolated granules” may differ in size, composition and structure from RNA granules that exist in the cell. Heterogeneity in RNA granule composition may also reflect the metabolic state of the granules and/or the existence of a variety of RNA transport granules with different *cis*-acting transport elements. We do not know how many different RNAs are transported by the A2 pathway and what percent of the RNA granule population they represent. Definitive identification of authentic RNA granule components requires co-localization analysis in intact cells. Key granule components identified by this approach include: A2RE RNAs, hnRNP A2, TOG, molecular motors, hnRNP E1, and components of the protein synthetic machinery [11,12,13].

RNA granules are transported along microtubules in oligodendrocyte processes and neuronal dendrites. RNA granules contain both plus end (conventional kinesin) and minus end (cytoplasmic dynein) motors and exhibit bi-directional transport in processes and dendrites. In oligodendrocyte processes microtubules are oriented predominantly plus end out which means that anterograde granule transport requires kinesin activity while retrograde transport requires dynein activity [16]. This was demonstrated by microinjecting function-blocking antibodies to different motor proteins into oligodendrocytes [17]. Injection of antibody to conventional kinesin inhibited anterograde transport resulting in accumulation of RNA granules in the perikaryon whereas antibody to dynein inhibited retrograde transport resulting in accumulation of granules in dendrites. In neuronal dendrites microtubules have mixed polarity so that bi-directional transport of granules can be achieved by either alternating activities of kinesin and dynein motors on the same microtubule or by granule switching among microtubules with opposite polarities. The presence of both plus end and minus end motors in granules, the bi-directional nature of granule transport along microtubules, and the mixed polarity of microtubules in dendrites of neurons, suggest that overall translocation of granules from perikaryon to dendrites is mediated by bi-directional motor-driven circulation of granules combined with local immobilization in dendrites rather than by directionally-biased transport along microtubules.

A2RE RNAs

Until recently it was not known if different RNAs are targeted to dendrites by independent pathways or if multiple RNAs follow the same pathway. However, studies in both oligodendrocytes and neurons indicate that several different RNAs share the same *cis/trans* trafficking determinants, are co-assembled into the same granules and are targeted to oligodendrocyte processes or neuronal dendrites by the same pathway.

In oligodendrocytes the only endogenous RNA for which *cis/trans* trafficking determinants have been characterized experimentally is MBP RNA (Figure 2). Deletion analysis of MBP RNA identified a tandemly duplicated 11 nucleotide *cis*-acting sequence that is necessary and sufficient for targeting of microinjected RNA to the myelin compartment of oligodendrocytes in culture [18]. This sequence, which was originally named the RNA transport sequence (RTS), was subsequently renamed the hnRNP A2 response element (A2RE) when it was found to bind to hnRNP A2 [19]. A2RE-like sequences have also been identified in the gag and vpr genes of HIV and shown to bind to hnRNP A2 and to be necessary and sufficient for dendritic targeting of microinjected RNA in oligodendrocytes. Gag and vpr RNAs microinjected into oligodendrocytes are co-assembled into the same granules and co-transported to the myelin compartment providing evidence for multiplexed dendritic targeting of different A2RE RNAs in oligodendrocytes [20]. Other oligodendrocyte RNAs that are localized to the myelin compartment also contain A2RE-like sequences and may follow the A2 trafficking pathway but these sequences have not yet been experimentally shown to bind to hnRNP A2 or to be necessary and sufficient for targeting to the myelin compartment.

In neurons α CaMKII, neurogranin (NG), ARC and PKM ζ RNAs are all localized to dendrites and all contain A2RE-like sequences that bind to hnRNP A2 and are necessary and sufficient for dendritic targeting of RNA in neurons (Figure 2). α CaMKII, NG and ARC RNAs have been shown to be co-assembled into the same granules and co-transported to dendrites by the A2 pathway providing evidence for multiplexed dendritic targeting of these A2RE RNAs in neurons [21]. Several other dendritically targeted neuronal RNAs also contain A2RE-like sequences and may follow the A2 pathway but A2RE-like sequences in these RNAs have not yet been experimentally demonstrated to bind hnRNP A2 or to be necessary and sufficient for dendritic targeting.

Comparison of A2RE sequences from different RNAs and species and mutagenesis of each base in the A2RE reveal that certain positions are more highly conserved and essential for dendritic targeting and hnRNP A2 binding while other positions are more variable and non-essential for hnRNP A2 binding and dendritic targeting [22]. The more highly conserved positions may represent contact sites for binding to hnRNP A2. Functional A2RE sequences cannot be identified by sequence analysis because many RNA sequences that appear similar to the A2RE do not bind to hnRNP A2 or mediate dendritic targeting. Definitive identification of functional A2RE sequences in different RNA requires: sequence similarity to previously identified A2RE sequences, high affinity binding to hnRNP A2 and deletion analysis showing that the sequence is necessary and sufficient for targeting to oligodendrocyte processes or neuronal dendrites. Given the fact that each of the dendritically targeted oligodendrocyte and neuronal RNAs that have been analyzed by these criteria have been shown to contain functional A2RE sequences it is probable that additional RNAs will be found to contain A2RE sequences that bind to hnRNP A2 and are necessary and sufficient for targeting to oligodendrocyte processes or neuronal dendrites.

Selection

If RNA molecules are targeted to oligodendrocyte processes or neuronal dendrites because they contain A2RE sequences then the selection machinery must recognize the A2RE sequence with sufficient specificity to discriminate A2RE RNAs from nonA2RE RNAs. The major protein in brain homogenate that binds specifically to A2RE sequences is hnRNP A2, a 36 kDa RNA binding protein consisting of two RNA recognition motif domains (RRM1, RRM2), followed by a glycine-rich dimerization domain (GRD) and an M9 nuclear localization domain [23, 24]. Alternative splicing of exon 2 near the N terminus and exon 9 in the GRD generates different isoforms (A2, A2b, B1, B1b). The three dimensional structure of hnRNP A2 has not been determined but a predicted structure for the region containing RRM 1 and 2 has been

generated by homology modeling with the corresponding region of hnRNP A1 [25]. Full-length hnRNP A2 forms oligomers in solution. Binding studies with synthetic oligoribonucleotides indicate that hnRNP A2 exhibits both specific binding to A2RE sequences and non-specific RNA binding [21,26]. Specific binding of hnRNP A2 to A2RE sequences could provide the basis for selection of A2RE RNAs for multiplexed dendritic targeting.

Individual RRM in hnRNP A2 bind more tightly to A2RE sequences than to non-specific RNA sequences. However, this may not provide sufficient selectivity for multiplexing because full length RNAs (both nonA2RE and A2RE RNAs) contain large numbers (thousands) of potential non-specific binding sites for hnRNP A2 while A2RE RNAs usually contain only a single sequence-specific A2RE binding site. One possible solution to this “needle in a haystack” problem is multivalent tethering of hnRNP A2 to non-specific binding sites on the RNA adjacent to the A2RE. Each hnRNP A2 molecule contains two RRMs and since the molecule dimerizes, the effective binding unit contains four potential RNA binding sites. Multivalent tethering of hnRNP A2 to non-specific sequences adjacent to the A2RE may reinforce binding of hnRNP A2 to A2RE RNA by increasing the effective on rate and/or decreasing the effective off rate for specific binding to the A2RE sequence, which could enhance selectivity for A2RE containing RNAs.

In both oligodendrocytes and neurons the concentration of hnRNP A2 in the nucleus is much greater than in the cytoplasm [27]. This means that in the nucleus newly synthesized RNAs (both A2RE and nonA2RE RNAs) are probably associated with multiple hnRNP A2 molecules through predominantly non-specific binding interactions. When the RNA is exported from the nucleus the lower hnRNP A2 concentration in the cytoplasm may favor specific binding of hnRNP A2 to A2RE sequences.

Studies in oligodendrocytes and neurons indicate that hnRNP A2 is co-localized with A2RE RNAs in ~ 75% of granules and with nonA2RE RNAs in ~ 25% of granules, indicating that the selection error rate is ~ 25% [21]. This error rate is higher than for other processes in gene expression such as transcription or translation suggesting that multiplexing is designed to maximize efficiency rather than fidelity. It may not be particularly deleterious to the cell if a small proportion of A2RE RNAs are not targeted to the myelin compartment or to dendritic spines or if a small proportion of nonA2RE RNAs are targeted to these subcellular compartments.

Linkage

Multiple different A2RE RNAs selected for multiplexing by binding to hnRNP A2 are linked together in granules. Linkage may be accomplished by multivalent binding of TOG protein to multiple hnRNP A2 molecules. TOG is a long (~ 218 kDa) flexible filamentous protein [28] that was shown to bind to hnRNP A2 in a yeast two hybrid screen and to co-localize with hnRNP A2 in RNA granules in oligodendrocytes [29]. TOG is also expressed in neurons where it may play a similar role (V. Tatavarty, unpublished results). Secondary structure algorithms predict that TOG is comprised of multiple clusters of HEAT repeats, called TOG domains. The structure of one of these TOG domains has been determined and other TOG domains are believed to have similar structures [30]. Each of the TOG domains can bind a dimer of hnRNP A2 [31]. Reducing expression of TOG in oligodendrocytes using RNAi reduces the amount of hnRNP A2 per granule [32] (Francone, V.P., unpublished observations). By binding to multiple hnRNP A2::A2RE RNA complexes each TOG molecule could serve as a nidus for granule assembly. The number of hnRNP A2 molecules bound to each TOG molecule may determine the linkage number (number of A2RE RNA molecules per granule) and bandwidth for multiplexed targeting of RNAs to oligodendrocyte processes or neuronal dendrites.

Studies in oligodendrocytes and neurons indicate that different A2RE RNAs are co-localized in ~70% of granules whereas A2RE and nonA2RE RNAs are co-localized in ~30% of granules. Thus the linkage error rate is comparable to the selection error rate. The selection error rate may contribute to the linkage error rate since linkage is mediated by TOG binding to hnRNP A2::A2RE complexes, which are formed by selection of A2RE RNA molecules by hnRNP A2. It is not known if some of the linkage error rate is due to non-hnRNP A2 RNA binding proteins binding to individual TOG domains. The relatively large linkage error rate underscores the relatively low fidelity of multiplexing in oligodendrocytes and neurons.

If TOG is a key component of RNA granules in oligodendrocytes and neurons it should be present in granules isolated from brain tissue. However, TOG was not identified in proteomic studies of isolated RNA granules [14,15]. In one study of RNA granules isolated from adult mouse brain [14] TOG (with a molecular mass of ~ 218 kDa) was not identified because only proteins with molecular mass < 100 kDa were analyzed. In another study of RNA granules isolated from embryonic day 18 rat brains [15], TOG was not identified because the processes of myelination and synaptogenesis, which involve dendritic targeting of TOG-containing granules in oligodendrocytes and neurons, respectively, are not maximally active until after birth. Technical difficulties that prevented identification of known granule components FMRP and Pur α/β proteins in this study may also have prevented identification of TOG.

Suppression

Multiplexing requires that translation of A2RE RNA molecules is suppressed during transport until the RNA granule reaches its final destination in the myelin compartment in oligodendrocytes or in dendritic spines in neurons. In oligodendrocytes translational suppression is mediated by hnRNP E1 binding to hnRNP A2 [33] (Figure 2). hnRNP E1 is an RNA binding protein that suppresses translation of lipoxxygenase RNA during erythrocyte development by binding, in conjunction with hnRNP K, to a *cis*-acting element termed the differentiation control element (DICE) [34,35]. This blocks recruitment of the 60S ribosomal subunit thereby inhibiting translation of lipoxxygenase RNA. DICE elements have not been identified in A2RE RNAs but hnRNP E1 binds to hnRNP A2 and suppresses translation of A2RE RNA in an hnRNP A2-dependent manner [33] suggesting that hnRNP E1 is recruited to A2RE RNAs by binding to hnRNP A2, thereby suppressing translation of multiplexed A2RE RNAs during transport. In the erythrocyte system, translation of lipoxxygenase RNA is desuppressed when hnRNP K is phosphorylated by src kinase causing the hnRNP E1/K complex to dissociate from the DICE [36]. In oligodendrocytes the mechanism whereby translation of A2RE RNAs is desuppressed when granules reach their final destination is not known but may involve dissociation of hnRNP E1 from the granule as a result of phosphorylation or some other post-translation modification in the myelin compartment in oligodendrocytes. A similar hnRNP E1-mediated mechanism may suppress translation of A2RE RNAs during transport in neurons as well.

Translation

The final step in the multiplexing process is demultiplexing, whereby individual A2RE RNA molecules in the granule are translated into proteins in the myelin compartment in oligodendrocytes or at dendritic spines in neurons. Since each granule contains multiple different A2RE RNAs one might expect that all the RNAs in the granule are translated simultaneously. However, careful serial section electron microscopic studies reveal that sites of myelin synthesis in oligodendrocytes and dendritic spines in neurons generally contain only one or at most a few polyribosomes [37,38]. Since each polyribosome represents a single translationally active RNA molecule this indicates that A2RE RNAs in granules are translated one at a time. Furthermore, since formation of a polyribosome requires repetitive re-initiation

of translation on the same RNA molecule this implies that translation initiation factors and ribosomal subunits engaged on one RNA are continually recycled back onto the same RNA. However, since each of the RNAs in the granule is presumably translated eventually, initiation factors and ribosomal subunits engaged on one RNA must occasionally migrate to engage different RNAs in the same granule. The temporal pattern of translation of different A2RE RNAs in each granule may be determined by *cis*-acting translational regulatory elements in individual RNAs.

Both MBP RNA in oligodendrocytes and α CaMKII RNA in neurons contain similar *cis*-acting elements that may regulate translation (Figure 3) [39]. The first is the cytoplasmic polyadenylation element (CPE). RNAs containing CPEs generally have short poly(A) tails, which is correlated with poor translation efficiency [40]. CPE is bound by the cytoplasmic polyadenylation element binding protein (CPEB). CPEB also binds maskin, which in turn binds the translation elongation factor eIF4E, inhibiting translation because eIF4E cannot bind eIF4G, which is an essential step for initiation of translation. Activation of the kinase Aurora A results in phosphorylation of CPEB, which causes it to recruit the cleavage and polyadenylation specificity factor (CPSF), which in turn recruits poly(A) polymerase that elongates the poly(A) tail [41]. Phosphorylation of CPEB also results in dissociation of maskin and eIF4E, which binds to eIF4G allowing translation initiation to proceed. Thus, elongation of the poly(A) tail is coincident with, and possibly related to translation activation [42,43]. The second translational regulatory element found in both MBP RNA and α CaMKII RNA is the A2RE, which binds to hnRNP A2, resulting in suppression of translation in the presence of hnRNP E1 [33] or stimulation of translation in the absence of hnRNP E1 (Figure 3) [44]. Binding of hnRNP A2 to TOG protein also appears to stimulate translation of A2RE RNAs because reducing expression of TOG with RNAi results in inhibition of translation of MBP RNA in oligodendrocytes. The reason why TOG stimulates translation of A2RE RNAs is not known although several possible explanations have been suggested [32]. One possible explanation is based on the function of TOG in linking multiple A2RE RNAs together in the same granule. By maintaining linkage among different A2RE RNAs in the same granule TOG may facilitate migration of translational initiation factors and ribosomal subunits among different A2RE RNAs resulting in enhanced translational output from each granule. In this way TOG may enhance translation of multiplexed A2RE RNAs in the myelin compartment of oligodendrocytes and in dendritic spines of neurons. Although CPE and A2RE are known to regulate translation of heterologous reporter RNAs in certain cell types it is not known if these *cis*-acting elements regulate translation of MBP RNA in oligodendrocytes or of α CaMKII RNA in neurons.

Consequences of multiplexing

Multiplexing results in coordinated targeting of ensembles of RNAs encoding functionally related proteins to the myelin compartment in oligodendrocytes or to dendritic spines in neurons. Linkage of multiple different RNAs in the same granule can lead to coordinated local translation of the encoded proteins, which may facilitate assembly of different proteins into complex structures or functional interactions among different proteins in complex pathways. This may be necessary for myelin synthesis in oligodendrocytes and synaptogenesis in neurons. Linkage of different RNAs in the same granule can also result in coordinated degradation of linked RNAs if ribonucleases are recruited to the granule. This may provide a mechanism for coordinated down regulation of ensembles of multiplexed RNAs, which may be important for terminating myelination in oligodendrocytes or for synaptic plasticity in neurons. Since the number of RNA molecules per granule is limited, multiplexing can also lead to competition among different A2RE RNAs for assembly into granules and dendritic targeting. For example overexpression of one particular A2RE RNA can inhibit dendritic targeting of other A2RE RNAs by competing for limiting multiplexing machinery. This could provide a mechanism for

altering the pattern of gene expression during myelin remodeling in oligodendrocytes or synaptic remodeling in neurons. Because of these potential consequences multiplexing may affect myelination in oligodendrocytes and learning and memory in neurons. This critical question could be addressed by disrupting multiplexing by creating transgenic mice where TOG is conditionally knocked out in oligodendrocytes or neurons. If such mice have defects in myelination or learning and memory, respectively, this would indicate that multiplexing is required for these important functions.

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References

1. Lecuyer E, Yoshida H, Parthasarathy N, Alm C, Babak T, Cerovina T, Hughes TR, Tomancak P, Krause HM. Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* 2007;131:174–187. [PubMed: 17923096]
2. Colman DR, Kreibich G, Frey AB, Sabatini DD. Synthesis and incorporation of myelin polypeptides into CNS myelin. *J. Cell Biol* 1982;95:598–608. [PubMed: 6183276]
3. Trapp BD, Moench T, Pulley M, Barbosa E, Tennekoon G, Griffin J. Spatial segregation of mRNA encoding myelin-specific proteins. *Proc. Natl. Acad. Sci. USA* 1987;84:7773–7777. [PubMed: 3478726]
4. Ghandour MS, Skoff RP. Double-labeling in situ hybridization analysis of mRNAs for carbonic anhydrase II and myelin basic protein: expression in developing cultured glial cells. *Glia* 1991;4:1–10. [PubMed: 1711012]
5. Gould RM, Freund CM, Barbarese E. Myelin-associated oligodendrocytic basic protein mRNAs reside at different subcellular locations. *J. Neurochem* 1999;73:1913–1924. [PubMed: 10537049]
6. Gould RM, Freund CM, Engler J, Morrison HG. Optimization of homogenization conditions used to isolate mRNAs in processes of myelinating oligodendrocytes. *Biol. Bull* 2000;199:215–217. [PubMed: 11081744]
7. Gould RM, Freund CM, Parlmer F, Feinstein DL. Messenger RNAs located in myelin sheath assembly sites. *J. Neurochem* 2000;75:1834–1844. [PubMed: 11032872]
8. Gorath M, Stahnke T, Mronga T, Goldbaum O, C. Richter-Landsberg C. Developmental changes of Tau protein and mRNA in cultured rat brain oligodendrocytes. *Glia* 2001;36:89–101. [PubMed: 11571787]
9. Lein ES, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 2007;445:168–176. [PubMed: 17151600]
10. Eberwine J, Belt B, Kacharmina JE, Miyashiro K. Analysis of subcellularly localized mRNAs using in situ hybridization, mRNA amplification, and expression profiling. *Neurochem Res* 2002;27:1065–1077. [PubMed: 12462405]
11. Barbarese E, Koppel DE, Deutscher MP, Smith CL, Ainger K, Morgan F, Carson JH. Protein translation components are colocalized in granules in oligodendrocytes. *J. Cell Science* 1995;108:2781–2790. [PubMed: 7593319]
12. Carson JH, Worboys K, Ainger K, Barbarese E. Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin. *Cell Motil.Cytoskeleton* 1997;38:318–328. [PubMed: 9415374]
13. Carson JH, Cui H, Krueger W, Schlepchenko B, Brumwell C, Barbarese E. RNA trafficking in oligodendrocytes. *Results Probl Cell Differ* 2001;34:69–81. [PubMed: 11288680]
14. Kanai Y, Dohmae N, Hirokawa N. Kinesin transports RNA: Isolation and characterization of an RNA-transporting granule. *Neuron* 2004;43:513–525. [PubMed: 15312650]
15. Elvira G, Wasiak S, Blandford V, Tons X-K, Serrano A, Fan X, del Rayo Sanchez-Carbente M, Servant F, Bell AW, Boismenu D, Lacaille J-C, McPherson PS, DesGroseillers L, Sossin WS.

- Characterization of an RNA granule from developing brain. *Mol. & Cell. Proteomics* 2006;5:635–651.
16. Lunn KF, Baas PW, Duncan ID. Microtubule organization and stability in the oligodendrocyte. *J. Neurosci* 1997;17:4921–4932. [PubMed: 9185530]
 17. Song J, Carson JH, Barbarese E, Li F-Y, Duncan ID. RNA transport in oligodendrocytes from the *taiep* mutant rat. *Mol. Cell Neurosci* 2003;24:926–938. [PubMed: 14697659]
 18. Ainger K, Avossa D, Diana AS, Barry C, Barbarese E, Carson JH. Transport and localization elements in myelin basic protein mRNA. *J. Cell Biol* 1997;138:1077–1087. [PubMed: 9281585]
 19. Hoek KS, Kidd GJ, Carson JH, Smith R. hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. *Biochemistry* 1998;37:7021–7029. [PubMed: 9578590]
 20. Moulund AJ, Xu H, Cui H, Krueger W, Munro TP, Prasol M, Mercier J, Rekosh D, Smith R, Barbarese E, Cohen EA, Carson JH. RNA trafficking signals in human immunodeficiency virus type 1. *Mol. Cell. Biol* 2001;21:2133–2143. [PubMed: 11238947]
 21. Gao Y, Tataavarty V, Korza G, Levin MK, Carson JH. Multiplexed dendritic targeting of α CaMKII, NG and ARC RNAs by the A2 pathway. *Mol. Biol. Cell.* in Press, published February 27, 2008 as 10.1091/mbc.E07-09-0914
 22. Munro TP, Magee RJ, Kidd GJ, Carson JH, Barbarese E, Smith LM, Smith R. Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. *J. Biol. Chem* 1999;274:34389–34395. [PubMed: 10567417]
 23. Siomi H, Dreyfuss G. RNA-binding proteins as regulators of gene expression. *Curr Opin. Genet. Dev* 1997;7:345–353. [PubMed: 9229110]
 24. Brumwell C, Antolik C, Carson JH, Barbarese E. Intracellular trafficking of hnRNP A2 in oligodendrocytes. *Expt. Cell Res* 2002;279:310–320.
 25. Landsberg MJ, Moran-Jones K, Smith R. Molecular recognition of an RNA trafficking element by heterogeneous nuclear ribonucleoprotein A2. *Biochemistry* 2006;45:3943–3951. [PubMed: 16548521]
 26. Shan J, Moran-Jones K, Munro TP, Kidd GJ, Winzor DJ, Hoek KS, Smith R. Binding of an RNA trafficking response element to heterogeneous nuclear ribonucleoprotein A1 and A2. *J. Biol. Chem* 2000;275:38286–38295. [PubMed: 11024030]
 27. Shan J, Munro TP, Barbarese E, Carson JH, Smith R. A molecular mechanism for mRNA trafficking in neuronal dendrites. *J. Neurosci* 2003;23:8859–8866. [PubMed: 14523087]
 28. Cassimeris L, Gard D, Tran PT, Erickson HP. XMAP215 is a long thin molecule that does not increase microtubule stiffness. *J Cell Sci* 2001;114:3025–3033. [PubMed: 11686305]
 29. Kosturko LD, Maggipinto MJ, D'Sa C, Carson JH, Barbarese E. The microtubule-associated protein, TOG, binds to the RNA trafficking protein, hnRNP A2. *Mol. Biol. Cell* 2005;16:1938–1947. [PubMed: 15703215]
 30. Al-Bassam J, Larsen NA, Hyman AA, Harrison SC. Crystal structure of a TOG domain: conserved features of XMAP215/Dis1-family TOG domains and implications for tubulin binding. *Structure* 2007;15:355–362. [PubMed: 17355870]
 31. Carson JH, Blondin N, Korza G. Rules of engagement promote polarity in RNA trafficking. *BMC Neurosci* 2006;7:S3. [PubMed: 17118157]
 32. Francone VP, Maggipinto MJ, Kosturko LD, Barbarese E. The microtubule-associated protein tumor overexpressed gene/cytoskeleton-associated protein 5 is necessary for myelin basic protein expression in oligodendrocytes. *J. Neurosci* 2007;27:7654–7662. [PubMed: 17634360]
 33. Kosturko LD, Maggipinto MJ, Korza G, Lee JW, Carson JH, Barbarese E. Heterogeneous nuclear ribonucleoprotein (hnRNP E1) binds to hnRNP A2 and inhibits translation of A2 response element mRNAs. *Mol. Biol. Cell* 2006;17:3521–3533. [PubMed: 16775011]
 34. Ostareck DH, Ostareck-Lederer A, Wilm M, Thiele BJ, Mann M, Hentze MW. mRNA silencing in erythroid differentiation: hnRNP-K, and hnRNP-E1 regulate 15-lipoxygenase translation from the 3' end. *Cell* 1997;89:597–606. [PubMed: 9160751]
 35. Reimann I, Huth A, Thiele H, Thiele BJ. Suppression of 15-lipoxygenase synthesis by hnRNP E1 is dependent on repetitive nature of LOX mRNA 3'-UTR control element DICE. *J. Mol. Biol* 2002;315:965–974. [PubMed: 11827469]

36. Ostareck-Lederer A, Ostareck DH, Cans C, Neubauer G, Bomsztyk K, Superti-Furga G, Hentze MW. c-Src-mediated phosphorylation of hnRNP K drives translational activation of specifically silenced mRNAs. *Mol. Cell. Biol* 2002;22:4535–4543. [PubMed: 12052863]
37. Barry C, Pearson C, Barbarese E. Morphological organization of oligodendrocyte processes during development in culture and *in vivo*. *Dev. Neurosci* 1996;18:233–242. [PubMed: 8911763]
38. Steward O, Falk PM, Torre ER. Ultrastructural basis for gene expression at the synapse: synapse-associated polyribosome complexes. *J. Neurocytol* 1996;25:717–734. [PubMed: 9023720]
39. Du I, Richter JD. Activity-dependent polyadenylation in neurons. *RNA* 2005;11:1340–1347. [PubMed: 16043499]
40. Preiss T, Muckenthaler M, Hentze MW. Poly(A)-tail-promoted translation in yeast: implications for translational control. *RNA* 1998;4:1321–1331. [PubMed: 9814754]
41. Barnard DC, Ryan K, Manley JL, Richter JD. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. *Cell* 2004;119:641–651. [PubMed: 15550246]
42. de Moor CH, Richter JD. Cytoplasmic polyadenylation elements mediate masking and unmasking of cyclin B1 mRNA. *EMBO J* 1999;18:2294–2303. [PubMed: 10205182]
43. Cao Q, Richter JD. Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J* 2002;21:3852–3862. [PubMed: 12110596]
44. Kwon S, Barbarese E, Carson JH. The cis-acting RNA trafficking signal from myelin basic protein mRNA and its cognate trans-acting ligand hnRNP A2 function to enhance cap-dependent translation. *J. Cell Biol* 1999;147:247–256. [PubMed: 10525532]

Figure 1. Oligodendrocytes and neurons form specialized processes and dendrites, respectively (A). Oligodendrocyte processes terminate in myelin-like membrane sheets. (B). Network of dendrites elaborated by a neuron in culture.

Figure 2. Multiplexing in oligodendrocytes and neurons

Panel A is a diagram of an oligodendrocyte illustrating multiplexed targeting of multiple different A2RE RNAs from the cell body on the left to myelin compartments on the right. Panel B is a diagram of a neuron illustrating multiplexed dendritic targeting of multiple different A2RE RNAs from the perikaryon on the left to dendritic spines on the right. Panel C is a schematic showing the different molecular components and processes involved in multiplexing.

Figure 3. Cis/trans determinants for translational regulation in MBP and α CaMKII RNAs
 MBP mRNA and α CaMKII mRNA contain analogous *cis*-acting translational regulatory elements: hnRNP A2 response element (A2RE), cytoplasmic polyadenylation element (CPE) and hexanucleotide (HEXA) that bind to cognate *trans*-acting translational regulatory factors: hnRNP A2 (A2), hnRNP E1 (E1), tumor over expressed gene (TOG), CPE binding protein (CPEB), maskin (MSK), eIF4E (4E), cleavage and polyadenylation specificity factor (CPSF). Binding of maskin to eIF4E RNA inactivates translation. Phosphorylation of CPEB facilitates recruitment of CPSF and polyadenylation of the RNA and also causes maskin to dissociate from eIF4E, facilitating recruitment of 40S ribosomal subunit to the RNA. Binding of hnRNP E1 to hnRNP A2 blocks recruitment of 60S ribosomal subunit preventing translation. Dissociation of hnRNP E1 from hnRNP A2 allows recruitment of 60S ribosomal subunit thereby activating translation.