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Analysis of gene expression in single live neurons

(amplified, antisense RNA/expression profile/mRNA complexity/pyramidal cell)

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ABSTRACT **We present here a method for broadly characterizing single cells at the molecular level beyond the more common morphological and transmitter/receptor classifications. The RNA from defined single cells is amplified by microinjecting primer, nucleotides, and enzyme into acutely dissociated cells from a defined region of rat brain. Further processing yields amplified antisense RNA. A second round of amplification results in $>10^6$-fold amplification of the original starting material, which is adequate for analysis—e.g., use as a probe, making of cDNA libraries, etc. We demonstrate this method by constructing expression profiles of single live cells from rat hippocampus. This profiling suggests that cells that appear to be morphologically similar may show marked differences in patterns of expression. In addition, we characterize several mRNAs from a single cell, some of which were previously undescribed, perhaps due to "rarity" when averaged over many cell types. Electrophysiological analysis coupled with molecular biology within the same cell will facilitate a better understanding of how changes at the molecular level are manifested in functional properties. This approach should be applicable to a wide variety of studies, including development, mutant models, aging, and neurodegenerative disease.**

Analysis of cellular diversity within the central nervous system (CNS) has been based largely on the presence of morphological and protein markers. However, individual cells may differ functionally based on their localization, synaptic and glial interactions, and the cadre of genes that are expressed. These factors combine to determine the cellular and functional identity of individual cells within the CNS. This interaction is so complex that the variety of cellular identities is certainly far greater than has been recognized to date using established methods.

The whole-cell patch-clamp techniques have provided a specificity and sensitivity of analysis that has permitted the characterization of the electrophysiological properties of single cells. However, little progress has been made in understanding the regulation of mRNA levels in individual cells. The limitation has been that most biochemical and molecular biological studies have used complex neural systems as sources of RNA. These include brain slices or dissected tissue samples and heterogeneous populations of cells grown in culture. Such preparations suffer from the large amount of tissue that is required for analysis, thereby making analysis of cellular specificity problematic. Although *in situ* hybridization (ISH) can be used to assess gene expression in individual fixed cells (1), this approach is limited in its ability to depict accurately the entire mRNA population of the cell. Furthermore, it has not been possible to obtain electrophysiological recordings from the individual cell of interest prior to the ISH. Examination of the entire mRNA population of a cell is an important aspect of cellular responsiveness since mRNA level changes often dictate changes in protein concentrations. If mRNA levels within the cell can be detected, then it may be possible to predict protein production and cellular responsiveness to exogenous stimuli.

The amount of mRNA within a single cell is estimated to be between 0.1 and 1 pg and as such is difficult to manipulate experimentally. The most commonly employed amplification procedure currently in use is the polymerase chain reaction (PCR) (2). Here, cDNA is first made from mRNA isolated from a single cell using reverse transcriptase followed by the addition of a primer site to the 3′ end of the first-strand cDNA (most commonly by the action of terminal transferase). The amplification process utilizes this added sequence and the poly(A) tail of second-strand cDNA as priming sites for the amplification process (3–5). PCR technology, however, suffers from several drawbacks that limit its utility for studies of gene expression in single cells. PCR works best when small regions of a few hundred nucleotides are being amplified. When larger cDNAs are amplified, there is a disproportionate decrease in the level of amplification such that longer cDNAs are not amplified at the same rate as shorter cDNAs (J.E., unpublished observations). In addition, the error rate of Taq polymerase (one mismatch for every 1000 bases of synthesis) will result in most PCR-amplified cDNAs containing several erroneous bases. Although these problems can be dealt with if anticipated, another concern is the difference in the efficiency of the amplification of different cDNA molecules (6) that can potentially result in several thousand-fold differential representation of these cDNAs in the cDNA population after as few as 30 rounds of amplification.

The technique of amplified, antisense RNA (aRNA) synthesis (7) circumvents many of these problems by facilitating the linear amplification of large mRNAs. The first step is the synthesis of an oligo(dT) primer that is extended at the 5′ end with a T7 RNA polymerase promoter. This oligonucleotide can be used to prime the poly(A)$^+$ mRNA population for cDNA synthesis. After the first-strand cDNA is synthesized, the second-strand cDNA is made using either "RNA nicking and priming" for RNA in solution or "hairpinning" for tissue sections (12). This is followed by a brief S1 nuclease treatment and "blunt-ending" with T4 DNA polymerase. The cDNA is now ready for amplification using the T7 RNA polymerase promoter (10, 11) to direct the synthesis of RNA. The RNA made using this technique is antisense to the poly(A)$^+$ RNA and can either be used as a probe or be cloned (12).

Although aRNA provides a means for amplification of RNA populations, it is extremely difficult to isolate RNA from a single cell. The primary obstacle to the isolation of RNA from a single cell is the tendency of RNA to interact

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Abbreviations: aRNA, amplified, antisense RNA; ISH, *in situ* hybridization.

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nonspecifically with plastic and glass. In this paper we report an approach to this problem that has enabled us to assess accurately the relative amounts of mRNAs for specific proteins and to demonstrate the expressed component of the genetic complement or "expression profile" of individual pyramidal neurons of the rat hippocampus. These experiments have been performed by injecting via patch pipette an oligo$(dT)_{24}$-T7 amplification oligonucleotide and reverse transcriptase into single, live acutely dispersed hippocampal cells. This method has previously been used in cultured fetal Purkinje cells (100-μm soma size) of the cerebellum (7).

MATERIALS AND METHODS

Electrophysiological Recording of Solitary Hippocampal Cells. Freshly dissociated single cells were obtained from the hippocampus of postnatal day 5–9 Long Evans rats (Charles River Breeding Laboratories) following a procedure originally designed for obtaining solitary cells from the rat retina (13). Many pyramidal cells could be identified based on morphological criteria since their soma and apical and basal dendrites remained relatively intact (see Fig. 1*A*). Conventional procedures were employed (14) using a model EPC-7 amplifier (List Electronics, Darmstadt, Germany) for whole-cell patch-clamp recording and intracellular dialysis. The recordings were done at room temperature (22–24°C) in a bath solution of physiological saline containing (in mM) 120 KCl, 0.5 $CaCl_2$, 1.0 $MgCl_2$, 5.0 EGTA, 10 Hepes, 0.2 (each) dATP, dGTP, dCTP, and TTP, 3.0 Na_2ATP, 2.0 Na_3GTP, and 0.01 sodium cGMP (pH 7.2). The typical range of pipette resistance, when filled with this solution, was 3–5 MΩ. Avian myeloblastosis virus reverse transcriptase (Seikagaku) at a final concentration of 1 unit/μl and oligo(dT)-T7 primer [AAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGCGC(T)24] at 1 ng/μl were included in the pipette solution to facilitate cDNA synthesis. The electrode was attached to the cell for times ranging from 1 to 20 min. The cellular contents were then aspirated into the patch pipette with a slight amount of suction. cDNA synthesis was continued in the patch electrode at 37°C for 60 min.

Amplification and Reamplification. Single-strand cDNA synthesis was followed by ethanol precipitation. The precipitated DNA was dissolved in 20 μl of H_2O and heated at 95°C for 3 min. Second-strand cDNA synthesis was accomplished as described (7) with the modification that 10 units each of T4 DNA polymerase and Klenow were used in the synthesis. After S1 nuclease treatment, end repair, and ethanol precipitation, the double-stranded cDNA was dissolved in 20 μl of Tris/EDTA (TE) and drop-dialyzed for 4 hr against 50 ml of TE. The DNA was recovered from the filter, and aRNA was made from DNA sample in 2-μl batches (7). The T7 RNA polymerase used in this reaction was in a concentration of 2000 units/μl; 2000 units were used per reaction. After phenol/chloroform extraction and ethanol precipitation, the aRNA (which can be used at this point for PCR or as a probe) was reamplified by dissolving the pellet in 20 μl of H_2O, adding 10–100 ng of random hexanucleotide primers (this will vary depending on yield of aRNA), 3 μl of 10× RT buffer (500 mM Tris, pH 8.3/1.2 M KCl/100 mM $MgCl_2$/0.5 mM $NaPP_i$), 3 μl of 100 mM dithiothreitol (DTT), dNTPs to 250 μM, H_2O to 27.5 μl, 0.5 μl of RNasin, and 2 μl of reverse transcriptase, and incubating at 37°C for 1 hr. This mixture was phenol/chloroform extracted and ethanol precipitated. The pellet was dissolved in 10 μl of H_2O, heat denatured at 95°C for 2 min, and quick-cooled on ice. Second-strand cDNA was synthesized by adding 100 ng of oligo(dT)-T7 amplification oligonucleotide, 2 μl of 10× KFI buffer (200 mM Tris, pH 7.5/100 mM $MgCl_2$/50 mM DTT/50 mM NaCl), dNTPs to 250 μM, H_2O to 17 μl, 1 μl of T4 DNA polymerase, and 1 μl of Klenow; this was followed by incubation at 14°C for 2 hr. After phenol/chloroform extraction and ethanol precipitation the pellet was dissolved in 20 μl of TE and drop-dialyzed, and aRNA was made as described. The level of incorporation of radioactivity was determined by binding of aRNA to DE-81 filters or by trichloroacetic acid precipitation.

ISH. Twenty-micrometer sections fixed in 4% paraformaldehyde and dehydrated through an alcohol gradient were prehybridized at 37°C for 3+ hr (normal ISH) or >12 hr (competition ISH) in 50% formamide, 600 mM NaCl, 10 mM Tris (pH 7.5), 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 1 mM EDTA, 100 μg of salmon sperm DNA per ml, and 5 μg of tRNA per ml. Terminal deoxynucleotidyltransferase was used at 37°C for 5 min to catalyze the addition of between 5 and 8 dATP[^{35}S]-labeled nucleotides to the 3′-hydroxyl terminus of oligonucleotide 11-2c (5′-TAACCAGCAAATCACTCTCTACAAATACAG-3′). The 3′-end-labeled oligonucleotide was then added to the section and allowed to hybridize overnight (>12 hr). Sections were then washed in a 2× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate)/20 mM sodium phosphate/0.05% $NaPP_i$ solution for 4 hr. A second wash consisted of 0.1× SSC/20 mM sodium phosphate/0.05% $NaPP_i$/10 mM 2-mercaptoethanol for 3–4 hr. Sections were air dried and exposed to Hyperfilm (Amersham) for 5 days. Emulsion dipping and developing of slides were as described (1).

RESULTS

We chose to work with acutely dissociated cells that exhibited morphological features characteristic of pyramidal cells—i.e., a large (15–30 μm) somal diameter, pyramidal- or oval-shaped soma with truncated apical and basal dendrites (Fig. 1*A*). Patch-clamp recording pipettes were filled with solution containing the amplification oligonucleotide and reverse transcriptase and, in the whole-cell patch-clamp recording mode, the contents readily dialyzed into these cells. Macroscopic inward and outward currents in response to depolarizing command potentials were monitored under voltage clamp (Fig. 1*B*). In separate experiments, we included Lucifer yellow in the pipette solution and determined that intracellular dialysis was complete by 3–5 min following rupture of the membrane. By the end of 5 min, the cytoplasmic contents were drawn into the recording pipette by applying negative pressure (suction). One of the distinct advantages of this procedure is that the components that direct cDNA synthesis are brought into immediate contact with the mRNA in a self-contained environment—namely, the cell and the patch pipette—which increases the efficiency of conversion of mRNA into cDNA.

A single round of aRNA amplification yielded ≈2000-fold amplification of the original mRNA. This calculation is based upon the amount of synthesized aRNA relative to that presumed to be present within a cell (0.1 pg). The aRNA at this point did not contain enough material to make a representative cDNA library and, furthermore, was difficult to use as a probe. To generate more aRNA, we reamplified the initial aRNA population. This was accomplished by using random hexanucleotides to prime cDNA synthesis from the aRNA by reverse transcriptase. Second-strand cDNA synthesis takes advantage of the fact that the cDNA has a poly(dA) tail. The oligo(dt)-T7 amplification primer can be hybridized to the cDNA and will serve as a primer for second-strand cDNA synthesis. While second-strand cDNA is synthesized from the oligo(dT) primer, the initial first-strand cDNA is extended at the 3′ end by copying of the antisense strand of the T7 promoter region of the amplification primer. In this manner, the T7 promoter is added to the aRNA population (see Fig. 2 for schematic). This second-round aRNA amplification routinely results in $>1 \times 10^6$-fold amplifications of the original starting material. An important

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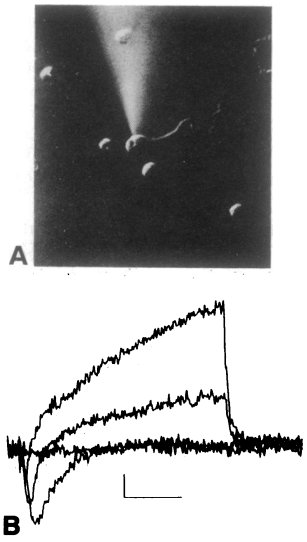

FIG. 1. Patch pipette introduction of cDNA synthesis reagents into single cells. (*A*) Phase-contrast photomicrograph showing the hippocampal cell prior to and during patch clamping. (*B*) Whole-cell current profile of the hippocampal pyramidal cell illustrated in *A*. Currents elicited in response to 20-mV depolarizing command steps from a holding potential of −60 mV are shown. (Vertical bar = 50 pA; horizontal bar = 5 ms.)

aspect of this procedure concerns the size distribution of the aRNA that is synthesized during the initial amplification procedure as well as in the second amplification. We have shown that the size distribution of the first-round aRNA is similar to the size of the original cDNA population (7). In Fig. 3, the sizes of first- and second-round radiolabeled aRNA populations are shown. Using this method of amplification, the second-round aRNA population will be smaller in size than the first-round population (size is dependent upon the amount of random primer), averaging ≈1 kilobase (kb). This population of aRNAs does not represent full-length aRNA for many endogenous mRNAs.

In studies of single pyramidal cells, the aRNA obtained from a second round of aRNA amplification was radiolabeled during synthesis and used as a probe to screen Southern blots containing known quantities of various cloned cDNAs (Fig. 4). These cDNAs were chosen for analysis because of the expectation that the mRNAs encoding these proteins would be present at differing abundances in hippocampal pyramidal cells. Other cDNAs could have been analyzed in the same manner. The relative intensities of hybridization within a blot have been assessed by scanning optical densitometry. The assessment of autoradiographic intensities of known mRNAs and comparison of this intensity to the intensity observed from other mRNAs is called "expression profiling." The rationale for this experiment rests on the assumptions that (*i*) the cDNA synthesis was constant across the population of mRNAs such that the abundance of any given type of cDNA is proportionate to the total cDNA population, as the original mRNA was to the mRNA population, and (*ii*) the aRNA amplification is linear and does not result in preferential amplification of certain RNA species over others. The first assumption has been addressed by several investigators and the results demonstrate that the cDNA population does, in fact, mirror the mRNA population. We have expanded upon initial observations by VanGelder and colleagues (7), with the result that there does not appear to be a significant difference in the amount of amplification of individual cDNAs into aRNA as demonstrated here. Since the cDNA population reflects the mRNA population, the aRNA should, likewise, reflect the abundance of the original mRNA population. Although we cannot exclude the possibility of preferential amplification of specific sequences, such differences in the amplification would be linear, not exponential (as with PCR). Additionally, approximate sizes of aRNA and cDNA have been normalized to exclude radioactivity differences in the probe. The expression profile of several different mRNAs within the aRNA population allows us to make comparisons among cells since the differences in efficiency of cDNA synthesis and aRNA

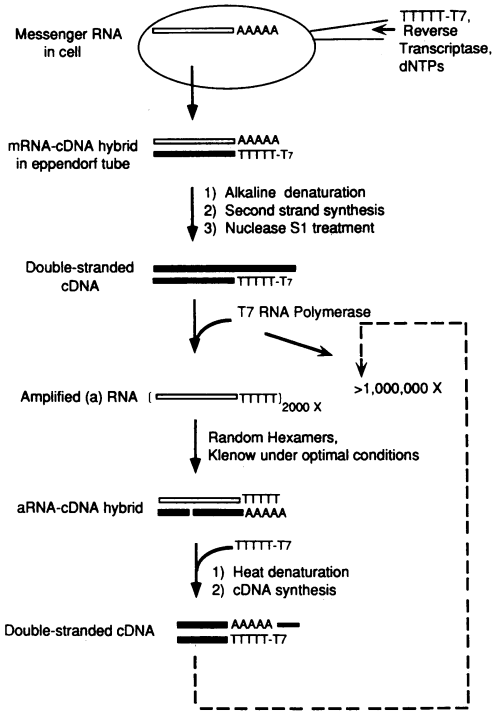

FIG. 2. Schematic of the reamplification procedure used to achieve a millionfold amplification of the original RNA population from a single cell.

amplification will serve to normalize the analysis across cells. Quantitation of individual species of aRNAs within the aRNA population has not yet been attempted.

We have analyzed the expression profile of several neurons from the rat hippocampus. Using the equivalent of 1/100th of the original starting material of RNA obtained from a hippocampal pyramidal neuron, we have shown the following rank order of mRNA abundance relative to neurofilament (1×): Ca^{2+} channel (3.4×) > γ-aminobutyric acid A receptor β-subunit (2.3×) > K^+ channel (0.54× and 0.58×) > c-*jun* (0.28×) > Na^+ channel (0.23×) and c-*fos* (0.22×), with undetectable levels of G_s, G_i, and glial fibrillary acidic protein (Fig. 4). The two different K^+ channel cDNA clones were used as an internal hybridization control since they will cross-hybridize. The similarity in their hybridization intensities suggests that the hybridization efficiency was consistent across the different cDNA clones. Indeed, the major difference between several morphologically similar neurons in which this analysis has been performed is a 10-fold variation in the ratio of Ca^{2+} channel to K^+ channel mRNA.

To assess further the complexity of the aRNA generated from a single neuron, we used radiolabeled aRNAs as a probe to identify cDNA clones from a cDNA library so that the complexity of the aRNA population could be determined. This was tested by randomly picking hybridizing cDNA clones of differing autoradiographic intensity from the li-

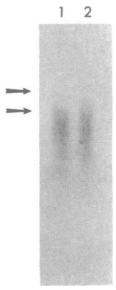

FIG. 3. Size distribution of aRNA population from a single hippocampal pyramidal cell: autoradiogram of a 1.4% denaturing agarose gel showing the autoradiographic distribution of aRNA from the first round of aRNA amplification (lane 1) and the second round of aRNA amplification (lane 2). The arrows indicate the positions of 28S (upper arrow) and 18S (lower arrow) rRNAs.

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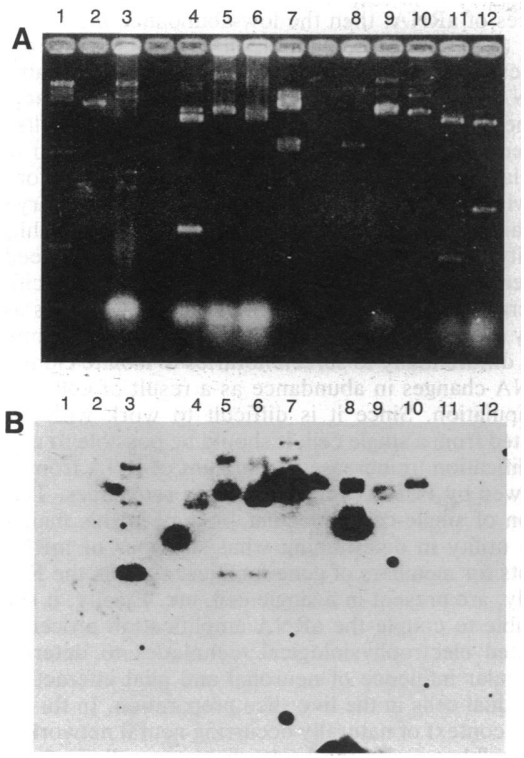

FIG. 4. Expression profiling of selected cDNAs. (*A*) Ethidium bromide-stained gel showing that nearly equivalent amounts of the indicated linearized plasmids were loaded on the gel. Lane 1, markers; lane 2, glial fibrillary acidic protein (GFAP); lane 3, neurofilament, light; lane 4, Na channel; lane 5, Kv1 channel; lane 6, Kv2 channel; lane 7, Ca channel; lane 8, γ-aminobutyric acid receptor; lane 9, c-*fos*; lane 10, c-*jun*; lane 11, G_s; lane 12, G_is. (*B*) Southern Blot of the gel shown in *A* after hybridization with radiolabeled aRNA from the equivalent of 1/100th of a single pyramidal cell. Note the low background: no hybridization to the marker lane (lane 1) and little hybridization to GFAP (lane 2). Hybridization was performed with 10 μg of oligo$(dT)_{36}$ in the hybridization solution. Lanes as in *A*.

brary. After plaque purification, the cDNAs were either plasmid-rescued or PCR amplified and cloned into the Bluescript vector. Upon screening of 40,000 cDNA clones from a NG108 cDNA library, 123 hybridizing plaques of varying autoradiographic intensity were isolated. Twelve of these clones were randomly selected to be sequenced and for those DNA sequences that were novel, abundance was determined by Northern blotting and ISH. This analysis revealed that 8 of the clones were derived from rRNAs, and 4 of the clones were encoded by mRNAs. The presence of ribosomal aRNAs in the aRNA population is not surprising, given that in making cDNA from total RNA there is always some rRNA converted into cDNA. Of the remaining cDNAs, one of them encoded ATP citrate-lyase (15) and the other three (11-2c, 11-2b, 24-2c) were novel, as determined by nucleic acid and protein data base searches. ATP citrate-lyase, which is present in every cell, has been shown to be moderately abundant in brain RNA. The novel cDNAs were random primed (8) and used to screen Northern blots containing total NG108 (9) and rat hippocampal RNAs. Hybridization of 11-2c and 24-2c was not detectable after a week-long exposure, whereas 11-2b hybridized to a band at ≈2.1 kb (data not shown). The lack of hybridization of 11-2c and 24-2c to Northern blots indicates that the mRNA for these molecules is present in NG108 cells and hippocampal tissue at <0.005% abundance. To determine cellular specificity and relative abundance of 11-2c in the rat brain, ISH was performed (Fig. 5). The film autoradiograms of coronal and saggital sections of the rat

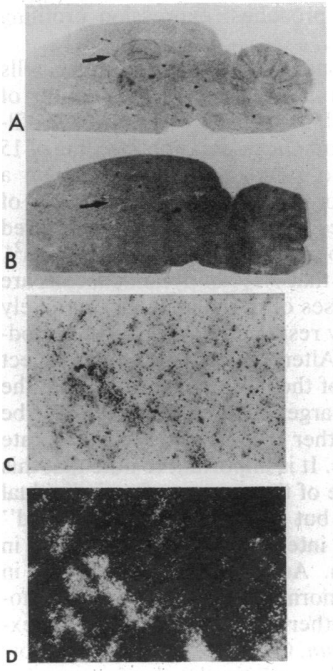

FIG. 5. ISH of clone 11-2c present within the single cell aRNA population. (*A*) Strong hybridization was apparent in the hippocampus (arrow), the tissue from which the aRNA was synthesized. (*B*) A 100-fold excess of nonlabeled oligonucleotide was hybridized to the section for 6 hr; this was followed by washing in 2× SSC at room temperature for 2 hr. (*C*) Bright-field view. (*D*) Dark-field view. Note the unlabeled nuclei (arrows) amidst the positive cytoplasmic signal. The slides were exposed at 4°C for 3–4 weeks.

brain through the hippocampus show that the highest level of expression of 11-2c mRNA is in the hippocampus (Fig. 5 *A* and *B*). The cerebellar hybridization is likely to be nonspecific because it was not competitively inhibited (Fig. 5*B*). Clone 11-2c showed signs of encoding a mRNA that is pyramidal cell specific (Fig. 5 *C* and *D*), 11-2b additionally hybridized to the striatal region, and 24-2c showed no apparent hybridization to other regions in this plane.

DISCUSSION

The quality of cDNA probes and libraries made from any tissue, in particular the brain, is determined by the number of distinct cDNA molecules in the population as well as the size of the cDNAs. Though the number of cDNA molecules in single-cell aRNA is large, the size is not full length. There are a number of potential reasons for this. It is possible that *in situ* the secondary and tertiary structures of the mRNA inhibit the processivity of reverse transcriptase along the mRNA. It is also possible that association of many mRNAs with cellular constituents such as ribosomes and the cytoskeleton may make it difficult for reverse transcriptase to make cDNA.

The aRNAs that are generated in this method are extended from regions containing polyadenosine, which means that most of the aRNAs are made from the 3′ end of the cellular mRNAs. Generation of aRNAs that span the entire mRNA sequence would require the use of random sequence oligonucleotides extended at their 5′ ends with the promoter sequence to prime cDNA synthesis. This would work best on isolated poly$(A)^+$ mRNAs. In the cell, the rRNAs would be primed by the random primer method of priming and subsequent amplification. Such vast amounts of ribosomal contamination would likely make it difficult to characterize the aRNAs corresponding to cellular mRNAs.

Regardless of potential limits to the size of aRNA from single cells, it is the consideration of aRNA complexity relative to initial mRNA complexity that is of greater importance in evaluating the usefulness of aRNA for assessing gene expression in single cells. Simply put, these issues of complexity relate to the number of unique RNA sequences in the original mRNA population of a cell and whether this is mirrored in the aRNA population. As this is a critical concept in this study, the complexity of the aRNA population has

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been assessed using two approaches: expression profiling and cDNA library screening.

This level of molecular analysis is dictated because cells are functionally classified on the basis of expression of specific marker proteins such as the expression of neurofilament protein. The results from the expression profiling of 15 pyramidal cells allows us to subclassify these cells on a functional basis derived from the ratio of expression of specific mRNAs within the cells. Two of these cells differed from the others in the ratio of expression of K^+ to Ca^{2+} channel mRNAs, suggesting that 7.5% of pyramidal cells are of this class. These two classes of pyramidal cells are likely functionally distinct and may respond to ionic or neuromodulatory signals differently. Alternatively, they may reflect different states of maturity of the neurons isolated from the postnatal hippocampus. A larger number of cells must be examined to determine whether this percentage is accurate over the whole hippocampus. It is important to note that this is not a quantitative measure of the amount of an individual mRNA, on a molar basis, but rather a "self-controlled" comparison of hybridization intensities of these channels in the same aRNA population. Any potential differences in amplification efficiency are normalized by the similar autoradiographic intensities of other molecules used in the expression profiling, such as c-*jun*. Overall, such data will allow us to generate a composite of the relative levels of expression of these and other important regulatory molecules within cells as a function of a wide variety of experimental manipulations, developmental events, or disease states.

As a technical note, as the cellular contents are aspirated into the patch pipette it is possible to watch the cell decrease in size. At the end of the aspiration procedure part of the cell membrane remains attached to the culture dish. If RNA is associated with the cellular membrane, then the cDNA that is made will not contain this material. The consistency of the experimental results (see discussion of Fig. 5) indicates that the amplification procedure is reliable, yet if there are classes of RNA (membrane, dentritic, axonal, etc.) that are not aspirated into the pipette, then they will not be represented in the aRNA population.

Though it is difficult to say that a given mRNA is absolutely specific (rather than enriched) in a certain cell type, the cDNA clone designated 11-2c by virtue of its ISH pattern is a candidate for being hippocampal cell specific in its expression. If this is true, then the gene for this mRNA may provide a cell-specific promoter region that would permit selected expression of downstream sequences in pyramidal cells of transgenic animals. Isolation of pyramidal cell-specific mRNAs, followed by promoter characterization, may permit a genetic approach to understanding pyramidal cell function.

Abundance of individual mRNAs is an inherent component of a mRNA complexity measurement. When RNA is isolated from a tissue, the abundance of any given RNA is reflective of RNAs within an individual cell as well as those that are present in multiple cells. Thus, complexity measurements of RNA isolated from tissues are not solely measurements of RNA distinctiveness but also a measurement of abundance (those RNAs that can be detected). This suggests that those RNAs that are present in high abundance will be more easily detected than will low-abundance mRNAs and that many of those mRNAs that are common to many cells (possibly serving a common function) are represented in higher abundance in a RNA population than mRNAs whose expression is limited to a single cell type. Of the 12 clones that were sequenced, 25% are low abundance, as determined by Northern blot analysis. Since it is probably easier to make cDNA from rRNAs than mRNAs in the single cell because of the size and availability for interaction with the primer, if the ribosomal clones are discounted in a comparison of abundances of aRNA, then the low-abundance mRNAs become 50% (2/4) of the population of sequences. These results suggest that it is, indeed, easier to isolate low-abundance mRNAs from a single cell than from a tissue homogenate.

The aRNAs that are made from individual cells can be cloned so that single-cell libraries from presumed different subclasses of hippocampal cells will be available for screening with radiolabeled probes. Although such a library will not contain full-length cDNA clones, it should be of high complexity. The aRNA that is made from single cells need not be cloned to be of use. The aRNA can be used as a specific probe to screen existing cDNA libraries to isolate clones as previously described. aRNA probes from different neurons can be used differentially to screen libraries to isolate clones whose mRNA changes in abundance as a result of cell identity or manipulation. Since it is difficult to work with the RNA isolated from a single cell, it should be possible to use aRNA amplification to increase the amount of RNA from the cell, followed by PCR to amplify specific sequences. This application of single-cell molecular biology in this manner may have utility in determining what subtypes of mRNA transcripts for members of gene families, such as the K-channel family, are present in a single cell, etc. Finally, it should be possible to couple the aRNA amplification procedure with selected electrophysiological techniques to determine the molecular influence of neuronal and glial interactions with individual cells in the live slice preparation. In this manner, in the context of naturally occurring neural networks, it may be possible to span the bridge between cellular functioning and molecular biology within the brain.

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