The successes and future prospects of the linear antisense RNA amplification methodology

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It has been over a quarter of a century since the introduction of the linear RNA amplification methodology known as antisense RNA (aRNA) amplification. Whereas most molecular biology techniques are rapidly replaced owing to the fast-moving nature of development in the field, the aRNA procedure has become a base that can be built upon through varied uses of the technology. The technique was originally developed to assess RNA populations from small amounts of starting material, including single cells, but over time its use has evolved to include the detection of various cellular entities such as proteins, RNA-binding-protein-associated cargoes, and genomic DNA. In this Perspective we detail the linear aRNA amplification procedure and its use in assessing various components of a cell's chemical phenotype. This procedure is particularly useful in efforts to multiplex the simultaneous detection of various cellular processes. These efforts are necessary to identify the quantitative chemical phenotype of cells that underlies cellular function.

Over the past ~25 years, particular emphasis has been placed on understanding the transcriptome of cells, with the knowledge that the fundamental player in converting genomic information into biological function is RNA. Whether translated into protein or functioning in a regulatory manner, RNAs underlie many aspects of cellular function, and it has become clear that their dysregulation is associated with various diseases. Understanding RNA biology requires detection, quantification, and sequence-specific characterization of the composite RNAs. The technical evolution of cell biology methods has resulted in the relatively easy analysis of RNA populations from organs and tissues where there are large amounts of RNA. However, transcriptomics analyses become problematic when smaller amounts of tissue, such as punch biopsies, are used for human diagnosis, or in examinations of individual single cells where RNA is present in small amounts and needs to be amplified to levels that can be experimentally analyzed.

For example, there are ~10,000 expressed genes in most cells[1], giving rise to 300,000 total mRNA molecules; thus the average number of RNA molecules is 30 per gene, with some genes transcribed into thousands of mRNAs, and others into fewer than ten. One way to detect such low-abundance RNAs is to amplify their numbers. The two most important considerations in the design of RNA amplification methods are the yield of amplification and how well the relative abundances of the different RNAs present in the original cell or tissue are maintained. The assumption is that the relative abundances of the amplified RNAs will reflect the biological state of the source, just as the endogenous RNA abundances do. To avoid systematic errors of uneven amplification of different RNAs that can occur with exponential amplification procedures (where error is also exponentially amplified), scientists developed linear nucleic acid amplification. Linear RNA amplification is an isothermal nucleic acid amplification system that rapidly amplifies target RNA populations. There are two primary linear amplification procedures in use today: linear aRNA amplification and rolling circle amplification. In this Perspective we concentrate on how the aRNA amplification methodology has been developed for a host of chemical analyses, so no further discussion of rolling circle amplification ensues.

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Linear aRNA amplification

Linear aRNA amplification is *in vitro* RNA-transcription-mediated amplification, and is also referred to as amplified RNA or simply aRNA. This technique was first developed and published in 1990 (ref. 2) as a method to amplify RNA populations, but since then it has been used for a number of other applications. In this procedure, Van Gelder *et al.*[2] first used reverse transcription to create a cDNA copy of the RNA template, incorporating an RNA polymerase promoter into each cDNA molecule by priming cDNA synthesis with a synthetic oligonucleotide (oligo(dT)-T7) that contained a poly(T) and phage T7 RNA polymerase promoter sequence. The poly(T) portion of the primer is used to select for mRNA species that contain a poly(A) tail, and the T7 promoter portion of the primer is used to direct T7 polymerase to bind to the promoter and synthesize an RNA copy of the cDNA template. This

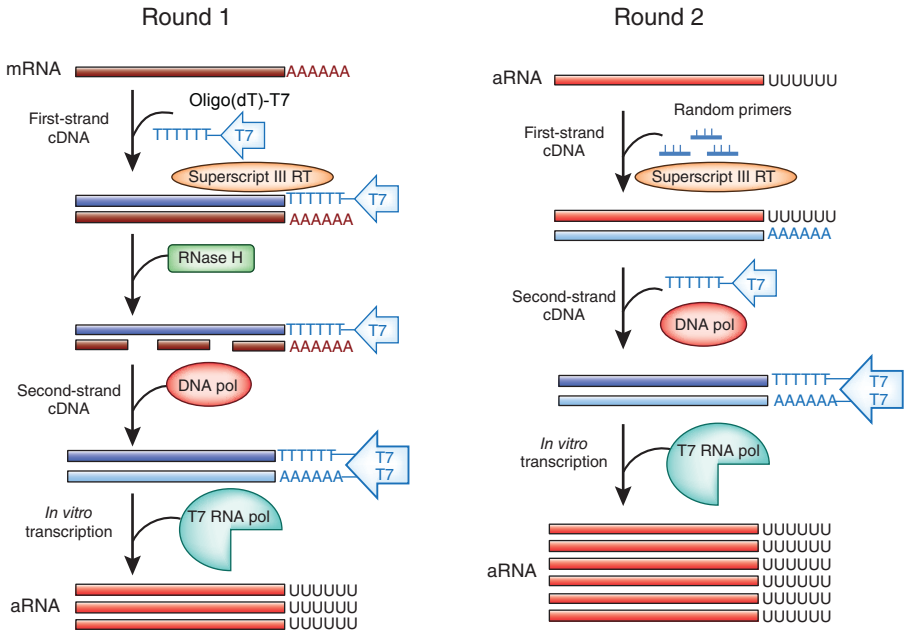
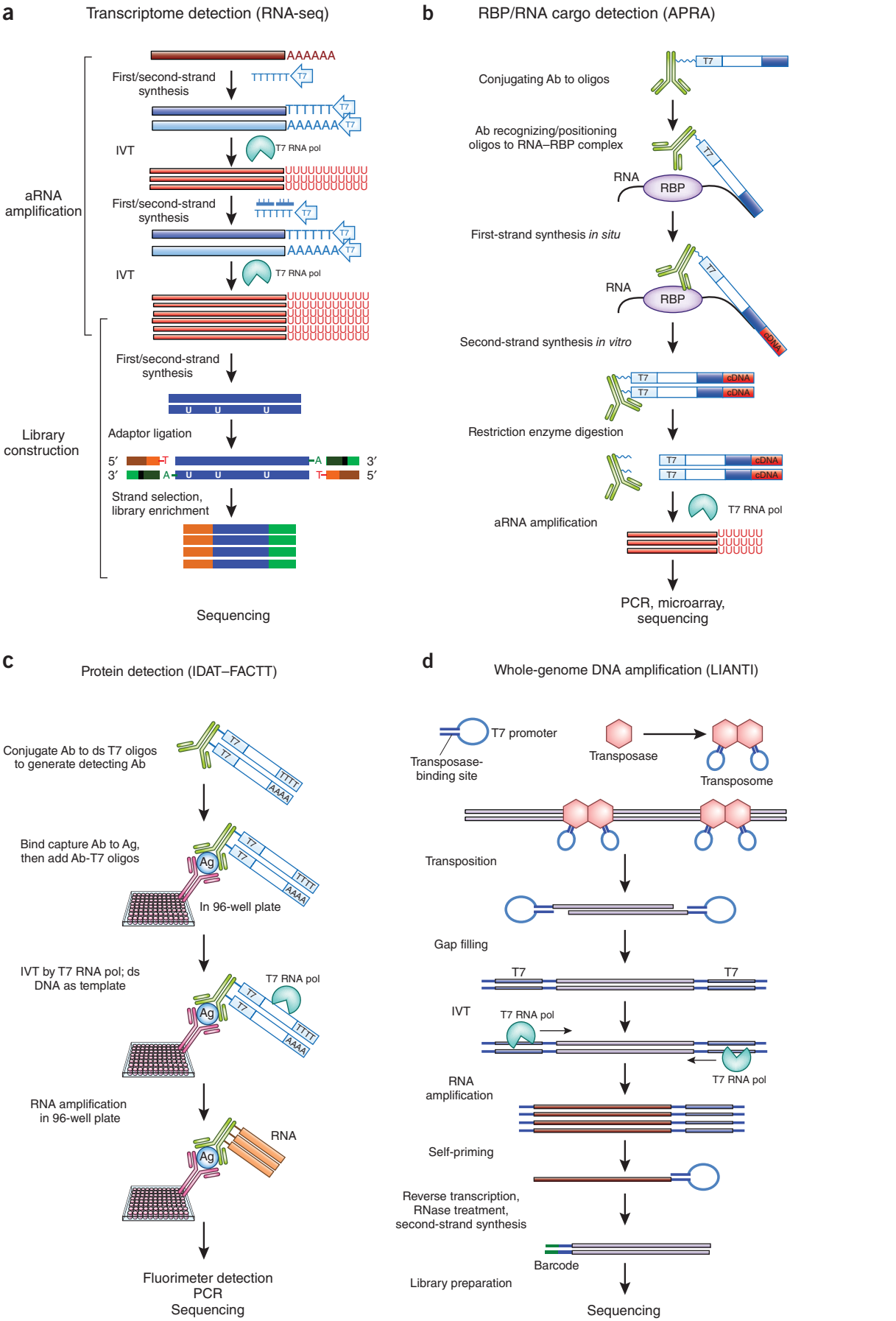


Figure 1 | Schematic linear antisense RNA (aRNA) amplification procedure. In the first round, first-strand cDNA synthesis by reverse transcription (RT) is primed from mRNA after oligo(dT)-T7 primer anneals to the poly(A) tail of mRNA. RNase H is then used to digest portions of the bound mRNA to create RNA fragments that serve to prime second-strand cDNA by DNA polymerase (pol). Finally, aRNA is amplified via linear *in vitro* transcription by T7 RNA polymerase, using the T7 RNA polymerase promoter incorporated in the double-stranded cDNA. In the second round, first-strand synthesis is primed by random primers instead of the oligo(dT)-T7 primer by reverse transcriptase using the aRNA as a template instead of mRNA. After RNA denaturation, second-strand synthesis is primed with the oligo(dT)-T7 primer, which binds to the poly(A) tail of the cDNA created during first-strand synthesis by DNA polymerase. Finally, RNA is again linearly amplified through the enzymatic activity of T7 RNA polymerase acting on its promoter that is incorporated into the double-stranded cDNA[2,3]. Credit: Marina Spence/Springer Nature.

occurs after synthesis of double-stranded cDNA, when T7 RNA polymerase is added and aRNA is transcribed from the cDNA template. There is then a second round of amplification, in which aRNA is amplified through conversion of aRNA into cDNA, with random hexanucleotide primers used to intitiate cDNA synthesis. Random primers are used because of their random annealing property, which allows them to prime any RNA species for reverse transcription into cDNA. The oligo(dT)-T7 primer serves as a primer for second-strand cDNA synthesis, in which the T7 promoter is added to the double-stranded cDNA at the end of the second round, thereby allowing subsequent second-round aRNA *in vitro* transcription by T7 RNA polymerase. If necessary, a third round of aRNA amplification can be performed after the second-round amplification procedure (**Fig. 1**).

After one round of amplification, ~2,000-fold quantities of original mRNA can be generated. After the second round, $>1 \times 10^6$–fold RNA amplification can be achieved[3], which is adequate for the generation of microarray probes[4] or a sequencing library[4,5]. This procedure has proven useful for amplification of RNA from single cells, which contain only ~10 picograms of total RNA[6–8]. Examination of expression profiles of single live cells has shown that linear aRNA amplification neither results in

Figure 2 | Schematic overview of applications of linear aRNA amplification for the detection of biological chemicals. (**a**) Transcriptome detection (RNA-seq). After two rounds of linear aRNA amplification from isolated single cells, an Illumina TruSeq library is generated as outlined. Because of the short length of the aRNA amplified by the procedure, the step that breaks long RNA into smaller parts in the original Illumina library protocol can be omitted. For strand specificity, deoxyuridine triphosphate is incorporated into the second-strand cDNA. After sequencing adaptor ligation, the cDNA fragments with adaptors at both end are PCR amplified, and ready for sequencing[7,8]. (**b**) RNA-binding protein (RBP)/RNA cargo detection (APRA). An antibody (Ab) to RBP is conjugated to an oligonucleotide and applied to fixed cells. After antibody–RBP binding, the oligonucleotide (containing the T7 promoter sequence) is positioned closely enough to the RNA to prime first-strand cDNA synthesis *in situ*. After second-strand synthesis *in vitro*, the antibody is removed by restriction enzyme cleavage and the aRNA is linearly amplified by *in vitro* transcription using the T7 promoter incorporated in the cDNA. The aRNA product is suitable for PCR, microarray, and next-generation sequencing analysis[43]. (**c**) Protein detection (IDAT). A detection antibody is first generated by conjugation of a target-protein-specific antibody to a double-stranded (ds) oligonucleotide containing T7 promoter. In a 96-well plate, a capture antibody then binds to the antigen (Ag) of interest from a sample. After the addition of detection antibody to the sample, RNA is linearly amplified by T7 RNA polymerase from the double-stranded oligonucleotide template incorporating the T7 promoter. The amount of RNA product is indicative of the original amount of antigen in the sample and can be used for fluorimeter detection, PCR. or sequencing[45]. (**d**) Whole-genome DNA amplification (LIANTI). A LIANTI transposon is first created by joining of the T7 promoter site to a transposase-binding site. This transposon is then mixed with transposase to generate the LIANTI transposome. After the LIANTI transposome is mixed with DNA isolated from single cells, the transposase mediates random insertion of LIANTI transposon into the DNA and subsequent excision of genomic DNA, which is followed by DNA polymerase gap extension. After the addition of T7 RNA polymerase, single-stranded aRNA is generated that is capable of self-priming on the 3′ end. After reverse transcription, RNase treatment, and second-strand synthesis, double-stranded LIANTI amplicons tagged with unique molecular barcodes are formed and ready for DNA library preparation and next-generation sequencing[11]. Pol, polymerase; IVT, *in vitro* transcription. Credit: Marina Spence/Springer Nature.



preferential amplification of certain RNA species (i.e., it shows no sequence preference) nor creates significant differences in the ratios of specific aRNAs in the amplified RNA population compared with those of the initial host cell mRNA abundances[3,9]. It should be noted that if there were bias in amplification, then the bias would be linearly amplified rather than exponentially as in PCR amplification. Furthermore, aRNA has been shown to provide an accurate and precise amplification product[9–12]. aRNA has been used extensively to generate probes for northern and Southern blotting, in tandem with PCR[13,14] and microarrays[4,12,15,16], and for sequencing of tissue-isolated RNA[7,10,17,18].

The high yield, accuracy, and precision (i.e., similarity of same-sample technical repetitions) of this transcription-based amplification system have led to its use as a reporter system to detect chemical moieties other than RNA. aRNA-associated reporter systems for proteins, RNA-binding protein cargoes, and genomic DNA sequences, as illustrated in **Figure 2**, benefit from the linear nature of the procedure and are further described in the following sections. Adaptations to the aRNA protocol that have enabled advances in the use of this technique to study other cellular chemicals are highlighted in **Table 1**.

aRNA for small-sample and single-cell transcriptomics

The first single-cell RNA analysis used aRNA (**Fig. 2a**) to identify ribosomal RNA in a single rat cerebellar Purkinje cell[2]. This was followed by the development of the first single-cell RNA-sequencing library, where aRNA was used on individual rat hippocampal neurons[3] and subsequently for differential display and Sanger sequencing of hundreds of RNAs[5] from single neuronal dendrites[18]. This showed that aRNA amplification was sufficiently sensitive to facilitate analysis of the RNA content of a well-defined subcellular region of a neuron—for example, a dendrite—independently from the cell soma[19,20], which represents a 1,000× larger volume. A timeline highlighting some of the major scientific advances made with aRNA is presented in **Figure 3**.

Microarray-based gene profiling was performed extensively before the advent of RNA-seq and is still used as an experimental tool for the direct monitoring of large numbers of mRNAs in parallel with RNA sequencing. The first transcriptomic-level evaluation of linear amplified aRNA for gene expression analysis by microarray was done by Lockhart *et al.*[12], and later Wang *et al.*[21,22] conducted a statistical assessment of aRNA performance in microarray analysis. Xu *et al.*[23] developed a human transcriptome microarray for high-throughput and low-cost expression-profiling analyses in clinical studies. With a small amount of human tissue as starting material, they linearly amplified nanogram amounts of total RNA, applied the aRNA to custom-designed 6.9-million-feature oligonucleotide arrays, and obtained reproducible and informative analyses of gene and exon expression.

The use of aRNA in single-cell analysis increased dramatically after RNA-seq was introduced. There have been many single-cell aRNA studies, but recently Spaethling *et al.*[8] identified >500 G-protein-coupled receptor mRNAs, including mRNAs for dozens of orphan G-protein-coupled receptors in serotonergic neurons, after three rounds of aRNA amplification and sequencing of RNA libraries. They also validated the presence of the receptors by voltage-clamp techniques. Using RNA deep sequencing, the same group also detected more than 12,000 expressed genes in primary cells from live neurosurgically resected adult human brains, uncovering cell-type-specific and patient-specific transcriptional hierarchies[7].

High-throughput linear aRNA amplification

CEL-seq is a modified aRNA method that allows for multiplexing of samples and high-throughput, next-generation sequencing[9]. Multiplexing is facilitated by alteration of the original aRNA primer so that it contains both a unique barcode and a 5′ Illumina sequencing adaptor between the anchored poly(T) and T7 promoter sequences. When aRNA is made via this procedure, it contains a barcode and a 5′ sequencing primer, which enables many cells to be barcoded and sequenced simultaneously. The Yanai group later adapted CEL-seq for use on Fluidigm's C1 system by adding unique molecular identifiers to the primer[24] that allowed RNA molecules to be counted. Later, Jaitin *et al.*[17] also reported the same approach, which they renamed massively parallel single-cell RNA sequencing (MARS-seq).

In an elegant study using CEL-seq, Hashimshony *et al.*[9] studied the embryonic development of *Caenorhabditis elegans* and reported differential distribution of transcripts between sister cells as early as the two-cell-stage embryo, identifying gene expression in a cell previously thought to be transcriptionally inert. Jaitin *et al.*[17] carried out multiplexing RNA-seq (MARS-seq) to sample thousands of mouse hematopoietic cells from the spleen, and uncovered cell-type heterogeneity in steady-state gene expression levels and regulated changes in RNA abundances after pathogen activation. Most recently, inDrop ('indexing droplets') sequencing technology was developed by Klein and colleagues[25,26]. This microfluidic approach encapsulates single cells in reaction droplets and barcodes the cDNA in each droplet during reverse transcription. Droplets are then pooled, and the RNA populations from all cells are linearly amplified and sequenced. This application of linear aRNA amplification was shown to exhibit low noise, be low-cost, and be effective in isolating and processing large numbers of individual cells simultaneously.

aRNA-based insights into human disease

Linear aRNA amplification has been used to study many human diseases. Its most prominent application has been to identify biomarkers, but it is also used to identify potential therapeutic targets. In many of the disease-associated studies, commercially available versions of the linear amplification technology have been used under trademarked names, including RiboAmp (Thermo), AmpliScribe (Epicentre), and MEGAscript (Invitrogen). Because aRNA is cell-type agnostic, it can be used in a variety of disease-associated cell types. For example, the transcriptome of platelets isolated from people with sickle cell disease[27] has highlighted the role of metabolic pathways in the disease. Transcriptomic insights into brain diseases such as schizophrenia[28], depression[29], Parkinson's disease[30], Alzheimer's disease[31], and even brain microvascular injury have provided many disease-associated targets for further analysis. The study of cancer has benefited

Table 1 | Linear aRNA protocol developments

Cellular function detection		Brief protocol description	Reference(s)
Transcriptome detection	Original aRNA procedure	1. One round of aRNA amplification developed and used for the amplification of single Purkinje neurons. Sequence heterogeneity assessed by northern blotting.	2
		2. Two rounds of aRNA methodology introduced to amplify mRNA from single live neurons.	3
	Subsequent modifications of aRNA procedure	1. *In situ* transcription with aRNA primer used to examine the mRNA populations in single neurons from fixed immunohistochemically labeled specimens.	19
		2. One or two rounds of aRNA labeled with dye, incorporating a cleavage step during *in vitro* RNA transcription.	12,15,16
		3. aRNA modified for template switching for better 5′-end representation in the aRNA products.	21
		4. Two rounds of aRNA adapted to amplify double-stranded CHIP-chip DNA fragments. After the addition of poly(dT) to the DNA template, oligo(dA)-T7 is annealed. The resulting double-stranded DNA fragment contains a functional T7 promoter that drives the *in vitro* transcription reaction, generating multiple RNA copies of the DNA template.	47
		5. aRNA modified for long SAGE (serial analysis of gene expression) for detection of large-scale gene expression from microdissected primary tissue. After one round of aRNA, first-strand cDNA is primed with SAGE random primer. Second-strand cDNA is then primed with Dynabeads oligo(dT) primer and used for standard SAGE library generation.	48
		6. aRNA modified for amplification on immobilized beads. After coupling of the oligo(dT)-T7 primer to magnetic beads, aRNA is synthesized directly on the beads to increase the yield.	49
		7. Modification of aRNA so that it produces sense RNA. The use of random primers containing an upstream T3 promoter sequence to prime second-strand cDNA synthesis enables *in vitro* transcription from T3 promoter sequences incorporated into the single-strand cDNA, thus producing amplified RNA in the sense orientation by T3 polymerase.	50
	High-throughput aRNA procedures	1. CEL-seq: a modified aRNA method that uses unique molecular indentifiers, developed for multiplexing of samples in high-throughput next-generation sequencing, scaled to Fluidigm's C1 single-cell isolation system.	9,24
		2. MARS-seq: a modified aRNA procedure essentially the same as CEL-seq.	17
		3. InDrop-seq: captures single cells along with a set of uniquely barcoded primers in droplets, enabling single-cell transcriptomics of a large number of cells.	26
		4. Automated microwell platform for high-throughput immobilized aRNA.	51
RBP/RNA cargo detection: antibody-positioned RNA amplification (APRA)		aRNA primer is conjugated to an antibody targeting RBP, thus concentrating aRNA primer near cargoes, allowing these RNAs to be transcribed into cDNA and linearly amplified.	43
Protein detection: immunodetection amplified by T7 RNA polymerase (IDAT)		1. IDAT: amplification of RNA from double-stranded oligonucleotides coupled to the antibody in the antibody–antigen complex enhances assay sensitivity at single-cell resolution.	45
		2. FACTT: a fluorescence-based modification of IDAT termed fluorescent amplification catalyzed by T7 polymerase techniques.	46
Whole-genome DNA amplification: linear amplification via transposon insertion (LIANTI)		Whole-genome amplification of single-cell genomic DNA using transposon-mediated aRNA linear amplification.	11

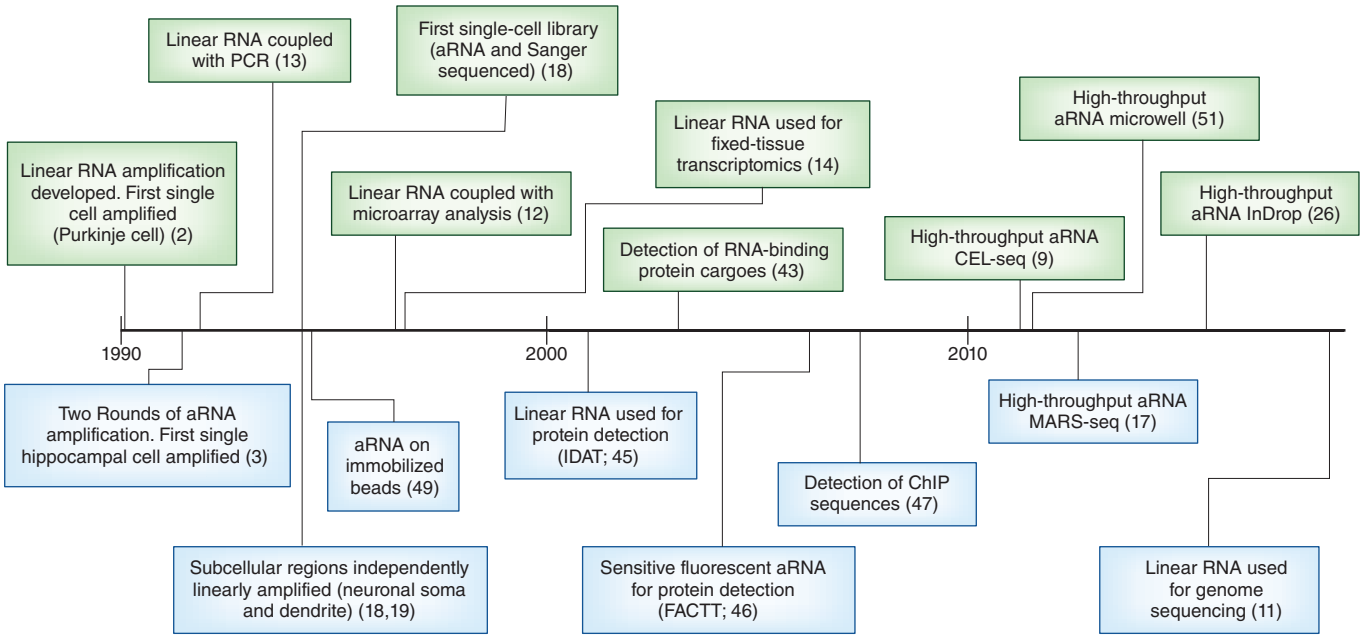


Figure 3 | A timeline of selected biological uses of linear aRNA amplification. The applications of linear aRNA amplification have steadily increased since its original publication in 1990[2]. There was a burst of technique development and applications during the five years after its initial publication, and a second burst of technique modifications and uses after 2010, when next-generation sequencing was introduced. This timeline presents selected highlights and is not exhaustive, as over this period thousands of papers were published on the use of linear aRNA amplification in different experiments (representative reference citations are shown in parentheses). Credit: Marina Spence/Springer Nature.

from aRNA analysis of a variety of cancers, including breast cancer[32], ductal cancer[33], liver metastases[34], prostate cancer[35], and various brain cancers[36,37]. An interesting application of linear aRNA amplification to the study of pancreatic cancer involved the identification of pancreatic tumor markers in saliva, suggesting that tumor-derived exosomes can provide salivary biomarkers of disease[38]. In addition, infection by pathogenic microorganisms has been identified in human samples by linear aRNA amplification, either directly through detection of microorganism-derived RNAs, or through biomarker surrogates in cases where changes in RNA abundance for host genes known to be modulated by infection are detected. This is exemplified by the detection of HIV[39], influenza[40], and severe acute respiratory syndrome (SARS) coronavirus[41] infections.

Whole-genome sequencing using aRNA

Most recently, linear aRNA amplification was used by Chen *et al.*[11] for whole-genome sequencing in a method called linear amplification via transposon insertion (LIANTI) (**Fig. 2d**). This procedure enables single-cell whole-genome amplification to facilitate sequencing of 95% of the single-cell genomic DNA, as well as detection of single-nucleotide variation with kilobase resolution. In LIANTI, the T7 RNA polymerase promoter sequence is incorporated into DNA associated with the T5 transposome, which, after insertion into the genomic DNA, embeds the T7 promoter into the transposon-flanked DNA. After the addition of T7 RNA polymerase, multiple RNA copies of the genomic DNA are synthesized. The LIANTI-produced aRNA is reverse-transcribed and made into a sequencing library for subsequent analysis. The high fidelity and yield of aRNA permits high-resolution detection of biological processes such as stochastic copying of DNA replication origins.

Identification of RNA binding protein–RNA cargo interactions using aRNA

The aRNA procedure has been adapted to identify mRNA targets that are associated with specific RNA-binding proteins (RBPs) (**Fig. 2b**). Miyashiro *et al.*[42,43] developed a technique called antibody-positioned RNA amplification (APRA), in which an RBP-specific antibody is conjugated to a single-stranded oligonucleotide containing a restriction enzyme site, a T7 RNA polymerase promoter sequence at its 5′ end, and a 15-base degenerate nucleotide sequence at its 3′ end. When the antibody–oligo conjugate is applied to fixed cells, the oligonucleotide acts as an APRA primer and is positioned near the RNAs that are associated with the RBP, and the degenerate sequence of the antibody-bound oligonucleotide can prime first-strand cDNA synthesis directly *in situ* in the fixed tissue section[44]. The antibody–cDNA complex is then removed from the fixed cells, and second-strand cDNA is synthesized *in vitro*. The double-stranded cDNA is removed from the antibody by restriction enzyme digestion, and aRNA is obtained by *in vitro* transcription followed by two rounds of aRNA amplification. Using the APRA procedure, the authors were able to isolate RNA cargoes associated with fragile X mental retardation protein (FMRP) and identify hundreds of previously unidentified putative FMRP targets. Among the FMRP APRA targets that were tested, >60% were confirmed as FMRP cargoes via filter binding or ultraviolet cross-linking assays to directly assess interaction with FMRP and mRNA targets[43].

Protein detection using aRNA amplification

The ability to detect antigens immunologically is often limited by the low affinity of many antibodies, the limiting amount of many antigens, and the high background often associated with high-sensitivity enzymatic or fluorescence detection methods. These issues can be overcome through the use of aRNA amplification for antigen detection rather than the standard fluorescence detection, as the protein-detection sensitivity can be increased ~10^9-fold over that of conventional ELISA methods. This aRNA protein-detection technique is termed immunodetection amplified by T7 RNA polymerase (IDAT) (**Fig. 2c**). Zhang *et al.*[45] developed a method to facilitate aRNA detection of antibody–antigen interactions by covalently attaching a double-stranded cDNA that contains a T7 RNA polymerase promoter in front of a reporter sequence to a specific antibody. After immunostaining with the IDAT antibody, RNA polymerase is added to the antigen–antibody complex to linearly amplify RNA from the cDNA that is conjugated to the antibody. Because the amplification is linear, the yield of aRNA is a direct measure of the amount of antibody–antigen interaction. A fluorescence-based modification of IDAT subsequently developed by the same group, termed fluorescent amplification catalyzed by T7 polymerase technique (FACTT), was successfully used to analyze low-abundance oncogene proteins in human serum[46]. Rather than using chemical cross-linking of the amplification primer to the antibody, FACTT uses streptavidin-labeled antibodies to bind the biotinylated amplification target sequence. Because streptavidin binds many biotinylated molecules with higher affinity than antibody–antigen binding, the sensitivity of FACTT is greater than that of IDAT. RiboGreen, a fluorescent RNA intercalating dye, can be used to quantify the yield of amplified RNA. IDAT and FACTT should be adaptable to automation and high-throughput protein screening.

The future of linear aRNA amplification

The coordinated activity of multiple cells gives rise to tissue-specific functioning. In this context the functional cell type is of utmost biological importance. Quantitation of the subcellular components and processes that give rise to cellular functions such as genomic DNA sequence variation, gene expression, methylation, and the creation of metabolites and proteins will improve the overall understanding of hierarchical cellular regulation and allow the construction of an 'architecture of cellular phenotype'. Such an architecture would enable the codetermination of genomic DNA sequence and expression state, as well as allow RNA and protein abundances to complement each other, with variations in dynamics and absolute and relative amounts of each cellular chemical resulting in specific cell-type designations. This will be possible only when all of these cellular constituents can be quantified in the same sample. The easy adaptation of the aRNA procedure to the analysis of different subcellular chemicals and biologies as illustrated above gives rise to the possibility of using this methodology in a multiplexed manner to simultaneously analyze multiple subcellular chemicals within a cell. Such multiplexing is currently possible for two chemicals at a time (for example, transcriptome and protein analysis), but adding further levels of analysis usually involves independent isolation of the additional chemical to be quantified, which risks differential loss of material and inaccurate interpretation of data. The linear aRNA reporting system, however, provides an opportunity to detect multiple biological entities in a single sample without specific isolation. This eliminates sample loss while harmonizing the detection output; because the RNA-polymerase-promoter-containing DNAs from the different detectors are transcribed, the resultant aRNAs can be used to deduce the proteome or other chemicals on the basis of the specific barcodes that are detected. For example, it may be possible to use APRA to detect specific RBP-associated RNA cargoes with unique barcodes in the same cell where the transcriptome is analyzed (both will be linearly amplified, but they will be distinct and distinguishable on the basis of different sets of barcodes). This would facilitate analysis of the dynamics of RNA movement between different subcellular compartments. As another example, when coupled with the use of antibodies to specific post-translational modifications such as phosphorylation or glycosylation groups, IDAT could be used to assess the biology and dynamics of such protein modifications at the same time as the transcriptome analysis. Careful use of barcodes in LIANTI-based DNA sequencing in conjunction with analysis of the transcriptome (using a distinctly barcoded oligo(dT)-T7 primer to prime amplification of the poly(A) RNA population) would allow analysis of both genomic DNA and the transcriptome in the same cell. One could take this even further by multiplexing more than two of these analysis procedures. For example, the transcriptome (assessed via the aRNA procedure), genomic DNA (analyzed by LIANTI), and protein (evaluated by IDAT) could all be analyzed in the same cell. Such combinatorial use of aRNA-amplification-based methods should enable the quantitative analysis of many cellular components and their associated biologies from a single sample, down to and including subregions of a single cell.

Given the flexibility of the technique and the ability of different aRNA reporting methods to barcode different chemicals, it is reasonable to presume that linear RNA amplification will continue to be used as a foundation for new technologies, providing deeper understanding of the multiple components and processes of cells and systems that contribute to their function.

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