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Yale University 1983 – 1987

Dr. Bobby Cherayil, 1986-1987 now at UMASS Boston
2 publications

THE EMBO JOURNAL

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Two RNA species co-purify with RNase P from the fission yeast *Schizosaccharomyces pombe*

Guido Krupp, Bobby Cherayil[1], David Frendewey[2], Satoshi Nishikawa[3] and Dieter Söll

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Communicated by W.Keller

RNase P activity from *Schizosaccharomyces pombe* co-purifies with two RNA species. These RNAs are associated with enzyme activity as judged by titrated micrococcal nuclease inactivation experiments. The two RNAs, K1- and K2-RNA, are 285 and 270 nucleotides long, respectively. Both RNAs are transcribed from one gene, present in a single copy in the haploid genome. The primary and a secondary structure of K RNAs have been determined and compared with M1 RNA, their counterpart from *Escherichia coli*. Very limited sequence homology was observed, and this agrees with the finding that no cross-hybridization with M1 RNA can be detected in a Southern analysis with yeast genomic DNA. However, the secondary structures of K RNA and M1 RNA show the same basic organization and one conserved local motif, the sequence GUG–AGGPu in an exposed hairpin loop.

Key words: tRNA processing/RNase P/*Schizosaccharomyces pombe*

Introduction

The processing of tRNA precursors to mature-size tRNA involves a complex set of different enzymes which act in a determined sequential order (Deutscher, 1984). Almost all tRNAs require endonuclease RNase P to generate their mature 5' termini by cleavage of tRNA precursors (Altman *et al.*, 1982). Two exceptions are known to date. In the formation of initiator $tRNA^{Met}$ from *Halobacterium volcanii* RNase P is not required as the nucleoside triphosphate of the initial RNA transcript is retained at the 5' end of the mature tRNA (Gupta, 1984). Another notable exception is the biosynthesis of eukaryotic $tRNA^{His}$ species where their 5'-terminal nucleotide is added after RNase P cleavage by a special guanylyl transferase (Cooley *et al.*, 1982).

An unusual characteristic of RNase P is the presence of an essential RNA component in the enzyme. This has been demonstrated in the RNase P from a variety of prokaryotic and eukaryotic organisms, including *Escherichia coli* (Stark *et al.*, 1978), *Bacillus subtilis* (Gardiner and Pace, 1980), *Saccharomyces cerevisiae* (Engelke *et al.*, 1985), *Schizosaccharomyces pombe* (Kline *et al.*, 1981), *Bombyx mori* (Garber and Altman, 1979) and mammals (Akaboshi *et al.*, 1980; Gold and Altman, 1986). Extensive studies on *E. coli* RNase P led to the striking discovery that under specific conditions *in vitro* the RNA subunit alone (M1 RNA) can catalyze the cleavage of tRNA precursors to produce mature 5' termini (Guerrier-Takada *et al.*, 1983). The complete nucleotide sequence and a secondary structure of M1 RNA are known (Guerrier-Takada and Altman, 1984). Homologous and heterologous reconstitution systems of active RNase P from inactive protein and RNA subunits have been developed for prokaryotic (Kole and Altman, 1979; Gardiner and Pace, 1980; Guerrier-Takada *et al.*, 1983) and eukaryotic systems (Gold and Altman, 1986).

To date eukaryotic RNase P enzymes are not yet characterized in detail. Due to their low abundance, difficulties in purification have hindered the isolation and characterisation of subunits from eukaryotic enzymes. In addition, the very low sequence homology between prokaryotic and eukaryotic RNA components (Gold and Altman, 1986) has prevented the identification of eukaryotic genes by cross-hybridization.

In a detailed study, we have monitored the RNA species which co-purified with RNase P activity from *S. pombe* during four column chromatographic fractionations. This revealed two RNA species, K1- and K2-RNA, which were always present in fractions containing enzyme activity. The functional importance of K RNAs for RNase P activity was supported by the results from titration inactivation of RNase P with micrococcal nuclease, since enzyme activity and the K RNA species were affected by similar nuclease concentrations. These RNA species and their gene have been characterized.

Results

Fractionation of RNase P and identification of RNA species

RNase P activities have been difficult to purify due to the presence of RNA and protein moieties in the enzyme (Altman *et al.*, 1982). Since the complex enzyme is disrupted by separations which use strong ionic interactions, we developed a purification scheme involving chromatographic fractionation by ion-exchange on the weakly basic DEAE–cellulose, gel filtration (Sephacryl S-300) and affinity chromatography (DNA–Sephacryl). Elution of RNase P from anion-exchange columns has proven to be a valuable first step in the purification of RNase P from all systems studied (Stark *et al.*, 1978; Engelke *et al.*, 1985; Kline *et al.*, 1981; Gold and Altman, 1986). We chose the same strategy in this preparation. As a substrate in our assays we used a synthetic tRNA precursor made by *in vitro* transcription with SP6 polymerase of a cloned *S. pombe* $tRNA^{Ser}$ gene. The resulting RNA contained a 28 base 5' flank.

S. pombe cell extract was passed over a DEAE–cellulose column. The RNase P activity eluted at a very low salt concentration (main peak at ~130 mM KCl) (data not shown) separating the bulk of the enzyme activity from material which contained large amounts of RNA. The second DEAE–cellulose chromatography removed most of the contaminating nucleic acids from the fractions containing the activity (Figure 1). The peak fraction of enzymatic activity (DE/2 fraction 13) contained eight RNA bands larger than tRNA size (Figure 1). No additional RNA species were observed when a 20-fold increased amount of RNA was analyzed (data not shown). This fraction was then used for all subsequent studies.

To get an estimate of the size of the *S. pombe* RNase P the

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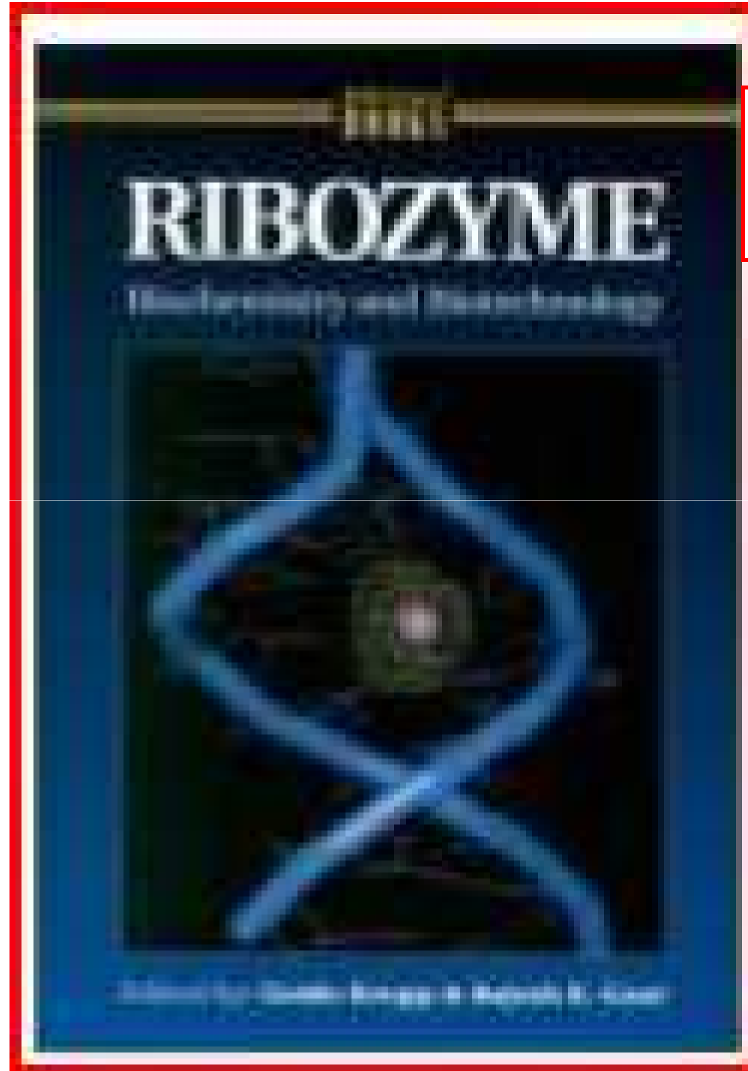
10 publications, 1 book



Ribozyme: Biochemistry and Biotechnology [Hardcover]

Guido Krupp (Author), Rajesh K. Gaur (Author)

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Nucleic Acids Research, 1993, Vol. 21, No. 1 21–26

Modification interference approach to detect ribose moieties important for the optimal activity of a ribozyme

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ABSTRACT

A new approach for modification interference studies is presented. It involves the use of phosphorothioates as a handle to analyze any desired base or sugar modification. This method was applied to identify ribose and phosphate moieties which could be important in the pre-tRNA recognition of *E.coli* RNase P RNA (M1 RNA). The utility of this technique was confirmed by detecting the inhibitory effect of a deoxyribose in the 5′-flank (position −1). This site was already known to interfere with RNase P cleavage, if modified. We have analyzed pre-tRNA^{Tyr} and pre-tRNA^{Phe} and found different interference patterns for both tRNAs. Two unpaired regions were involved in both pre-tRNAs. Phosphorothioates interfered at the transition between acceptor- and D-arms. The results with deoxythymidines in the T-loop indicated that deoxyribose moieties or the extra methyl group in thymidine could interfere with RNase P cleavage. These data suggest that even in complete pre-tRNAs, only a few intact ribonucleotides are important in the substrate recognition by RNase P. We have demonstrated the potential of this new approach which offers many future applications in all fields involving nucleic acids, for example RNA processing, action of ribozymes, tRNA charging and studies related to DNA promoter recognition.

INTRODUCTION

Chemically synthesized RNAs which contain modified nucleotides at a few specific positions have been used to stabilize the RNAs and to elucidate the importance of these nucleotides in various studies. Prerequisites for such strategies are, first, the characterization of nuclease-resistant modifications, like phosphorothioates, or 2′-modified ribose moieties; second, the identification of the positions where modifications can be introduced into an oligonucleotide without loss of its activity. Unfortunately a serious drawback of this approach is the difficulty to predict all sites which can be modified without severely effecting the catalytic activity of the ribozyme. This is a cumbersome analysis even for small RNAs, as experienced by others, and it will be difficult to draw a final conclusion, unless all positions are analyzed independently (1−6). Moreover, for large RNAs this approach will be more tedious or even impossible; already transfer RNAs are refractory. A powerful alternative could be the modification interference analysis (7−11). However, in most of the cases this approach requires methods for the detection of modified sites in the nucleic acid which is impossible for many modified nucleosides, like m^5C.

We wish to report here a modification interference approach which can exploit phosphorothioates as a tool to analyze any kind of nucleoside modification in RNA and DNA. It is based on the presence of a low level (about 5-10%) of the modified nucleotide in enzymatically synthesized RNAs. The modification of interest is incorporated into an RNA transcript using an appropriate mixture of normal nucleoside triphosphates together with a modified NTPαS. As a result, each position with a modified base (or sugar) is next to a labile thio-substituted phosphodiester bond. In this fashion, any modification in sugar or base moieties can be combined with a scissile nucleic acid backbone; useful for both, RNA and DNA.

As an example we analyzed which ribose moieties in pre-tRNAs should remain intact for efficient cleavage by RNase P. RNase P is a ribonucleoprotein which makes a single endonucleolytic cut in the pre-tRNA forming the mature 5′-terminus of the tRNA molecule (12). Protein and RNA components of RNase P are essential *in vivo* (13), however, *in vitro* the eubacterial RNA component acts as a true enzyme (ribozyme) and it is able to cleave tRNA precursors at the correct site (14,15). In our study we used the *E. coli* RNase P RNA (M1 RNA).

Towards this end, we used dTTPαS and dATPαS to locate 2′-OH moieties which must remain intact for efficient pre-tRNA processing by RNase P. Obviously, one prerequisite of this analysis is the possibility to perform enzymatic RNA synthesis with the modified NTPαS. However, only dATPαS and dTTPαS were efficient substrates for T7 RNA polymerase (more details about the use of T7 RNA polymerase and 2′-deoxy- or 2′-O-methyl NTPαS will be published elsewhere).

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Rapid kinetic characterization of hammerhead ribozymes by real-time monitoring of fluorescence resonance energy transfer (FRET)

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ABSTRACT

In established methods for analyzing ribozyme kinetics, radiolabeled RNA substrates are primarily used. Each data point requires the cumbersome sampling, gel electrophoretic separation, and quantitation of reaction products, apart from the continuous loss of substrate by radioactive decay. We have used stable, double fluorescent end-labeled RNA substrates. Fluorescence of one fluorophore is quenched by intramolecular energy transfer (FRET). Upon substrate cleavage, both dyes become separated in two RNA products and fluorescence is restored. This can be followed in real time and ribozyme reactions can be analyzed under multiple (substrate excess) and under single (ribozyme excess) turnover conditions. A detailed comparison of unlabeled, single, and double fluorescent-labeled RNAs revealed moderate kinetic differences. Results with two systems, hammerhead ribozymes in I/II (small ribozyme, large substrate) and in I/III format (large ribozyme, small substrate), are reported.

Keywords: BODIPY; Cy-5; ethanol; FAM; fluorescein; fluorescence; quenching; rhodamine; TAMRA

INTRODUCTION

Hammerhead ribozymes are small RNA motifs that self cleave at one specific phosphodiester bond. In vivo, this ribozyme occurs as an intramolecular motif in several small RNA pathogens, in viroids, and in virusoids (Symons, 1989). In vitro, it can be split into two RNA oligonucleotides by opening two of the three hairpin loops in the minimal conserved structure (Fig. 1A) (Uhlenbeck, 1987; Haseloff & Gerlach, 1988; Koizuma et al., 1988; Jeffries & Symons, 1989).

Many features of RNA biochemistry were studied with these small catalytic RNAs, including mutational analyses with both natural and modified nucleotides (Bratty et al., 1993; Burgin et al., 1996; Clouet-D'Orval & Uhlenbeck, 1996), the functional importance of metal ions (Dahm & Uhlenbeck, 1991; Murray et al., 1998), and high-resolution structures with X-ray crystallography (Pley et al., 1994; Scott et al., 1995) and with intramolecular fluorescence resonance energy transfer (FRET) (Tuschl et al., 1994). Furthermore, format I/III is vigorously pursued as a tool for specific gene inactivation (Haseloff & Gerlach, 1988; Rossi, 1997).

All experiments that employ a ribozyme require kinetic analysis of the cleavage reaction. This dynamic process has to be analyzed by data acquisition at multiple time points—a rather cumbersome requirement. Furthermore, radiolabeled substrate RNAs are primarily used, and this requires repetitive RNA preparation and purification. Recently, real-time measuring of changes in FRET was introduced to follow substrate–ribozyme interactions (Li et al., 1995; Perkins & Goodchild, 1997; Walter & Burke, 1997). Substrate RNAs were applied that carried only one fluorescent label and changes in FRET occurred upon formation of the intermolecular complex with ribozyme RNA. These studies revealed details in substrate binding of the *Tetrahymena* group I ribozyme (Li et al., 1995). For hammerhead (Perkins & Goodchild, 1997) and hairpin (Walter & Burke, 1997) ribozymes, real-time monitoring of the subsequent cleavage reaction was possible. Fluorescence of a fluorescein-labeled substrate was quenched in the complex with the ribozyme due to interaction with a quencher dye (Perkins & Goodchild, 1997) or with a proximal, unpaired G base (Walter & Burke, 1997) in the ribozyme. Because monitoring of cleavage kinetics was dependent on the preformed ribozyme–substrate complex, it was limited to single-turnover conditions with saturating ribozyme excess. Similar to the 5′-exonuclease assay for real-time mon-

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Dr. Kumud Singh, Ph.D. at Dehi Uniersity, now at Scripps Institute
8 publications

Cooperation initiated: University Kiel & artus GmbH

Dr. Sujala Kapur, Safdarjang Hospital, Delhi, India
2 publications

Reliability of Detecting rRNA Sequences of *Chlamydia trachomatis* With Fluorescence in Situ Hybridization Without Amplification

Sujala Kapur, M.D., Margoob Ahmed, M.Sc., Vineeta Singh, Ph.D., Guido Krupp, Ph.D., Sudha Salhan, M.D., and Aruna Mittal, Ph.D.

Objective

To use fluorescence in situ hybridization (FISH) using ribosomal RNA (rRNA) oligonucleotide probes as the target nucleic acid for the detection of Chlamydia trachomatis.

Study Design

Suitable sequences selected from the rRNA sequence of C trachomatis *were labeled with a fluorescence dye and used in FISH for detecting chlamydial inclusion bodies and/or elementary bodies in paraformaldehyde-fixed urogenital swab samples. The sensitivity and specificity of the FISH assay were compared with those of the polymerase chain reaction (PCR) using plasmid primers. Positive known* C trachomatis*–infected McCoy cells were used as positive controls. Urogenital swab specimens that were C trachomatis negative on culture and PCR were used as negative controls.*

Results

Among the 128 samples included in the study, FISH was positive in 28 (21.8%) and PCR in 33 (25.7%). A significant correlation was found between the 2 detection methods. Results of PCR and FISH were consistent in 115 of the 128 samples (R=0.89). Thirteen samples showed discordant results. Of these, 9 FISH negative samples were PCR positive and 4 FISH positive samples were PCR negative.

Conclusion

FISH was a highly specific and fairly sensitive technique for detecting C trachomatis. *Signal amplification techniques and use of different fluorophores may further increase the sensitivity of this technique.* (Acta Cytol 2006; 50:277–283)

FISH has the potential to provide rapid and unequivocal identification of such organisms as* C trachomatis*, which are difficult to culture.

Keywords: *Chlamydia trachomatis*; 16S ribosomal RNA; hybridization, fluorescence in situ; polymerase chain reaction.

The use of probes that target ribosomal RNA (rRNA) makes direct detection of single cells possible without amplification. This phylogenetic stain-

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Dr. Krupp is Director, Artus-Biotech.

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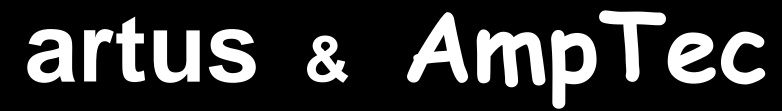
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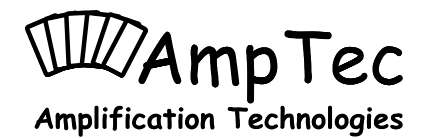
the PCR reference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What you should know about the companies artus & AmpTec

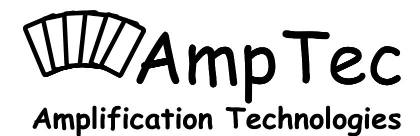
Company Headquarters in Hamburg

- 1998: Foundation of artus GmbH –
  as a spin-off from the Bernhard-Nocht-Institute for Tropical Medicine
  6 founders: BNI Hamburg – Bremen University – Kiel University

- 2000: First positive BSE case in Germany ever – detected at artus

- 2003: The first available RT-PCR kit for “SARS virus“ HPAC
- 2003: artus acquired general IVD PCR license from Roche
- 2003: Launch of ExpressArt mRNA amplification kits

- 2005: artus-section with IVD PCR kits was acquired by Qiagen
- 2005: Transfer of the ExpressArt product line to **AmpTec GmbH**



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AmpTec GmbH & India & USA

Ocimum Biosolutions

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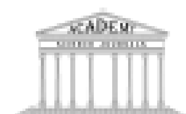

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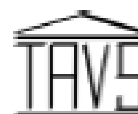

Original Article

Seasonal Changes of Gene Expression in Roe Deer (*Capreolus capreolus*) Testis Measured by Expression Microarray Analysis

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Abstract

In seasonal breeders such as the European roe deer (*Capreolus capreolus*) the well timed regulation of testicular functions is crucial to reproductive fitness and successful breeding. Such functions are in particular the production of spermatozoa and testosterone. Having only a very short rutting season from mid-July to mid-August, roe deer testis undergo drastic cyclic changes during the transitions between breeding and non-breeding seasons. These changes require mechanisms to stimulate both cell proliferation and spermatogenesis during testis growth (recrudescence) and to initiate repression of spermatogenesis during testis involution. The expression of genes responsible for these local control mechanisms needs to be seasonally regulated. Due to the lack of a fully sequenced roe deer genome, we used an 8K expression microarray from cattle as its evolutionary closest and sequenced relative to investigate the gene expression changes in roe deer testis during the transition between the two extreme phases of the circannual testicular development: the arrest of spermatogenesis (December) on one hand and the fully activated spermatogenesis (June) on the other. During the transition from December to June, 622 genes were significantly differentially expressed ($p < 0.05$). Out of the 407 genes found to be up-regulated, 197 exhibited an increase in expression of twofold or more, and 132 out of the 215 down-regulated genes showed a decrease of twofold or more. Based on database queries, 51 of the differentially expressed genes could be assigned to 50 broad biological processes, 32 of which covered molecular functions and 28 were assigned to different cell components.

Key words: reproduction, spermatogenesis, circannual rhythm, regulation

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INTRODUCTION

Many mammalian species that are distributed over a wide range of habitats, have evolved numerous adaptations in order to cope with the seasonally changing conditions in their distribution range (Fickel et al., 1999; Fickel and Reinsch, 2000). For the European roe deer (*Capreolus capreolus*) these environmental conditions can vary considerably, ranging from very hot and dry periods in southern Europe (Spain, Italy) to extreme cold periods in northern Europe (Scandinavia, Russia). Due to the seasonally varying types and availabilities of nutrition, these adaptations have to include habitat selection and special feeding strategies to ensure both sufficient energy metabolism (Duncan et al., 1998; Fickel et al., 1998; Clauss et al., 2008; Hofmann et al., 2008) as well as seasonally driven changes in locomotion, behavior and reproduction (Andersen et al., 1998). In roe deer the latter is characterized by a distinct seasonal pattern with a fully arrested

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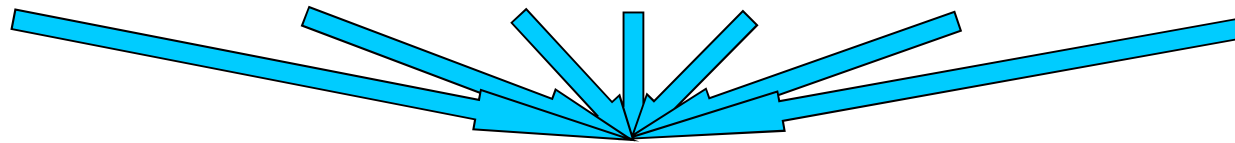
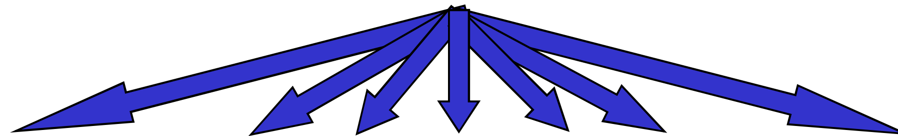
Many Applications

Tissues/Cells/Body Fluids – (Needle) Biopsies – (Laser) Microdissection – Sorted (Stem) Cells

Degraded RNA – FFPE RNA – Bacteria



RNAready isolation kits
&
ExpressArt mRNA Amplification kits



Many Microarray Platforms & qPCR & NGS

Flourescence — **Agilent**

Biotin — **Affymetrix, Illumina, Axela**

ExpressArt kit for mRNA Amplification

Pico-version

Ryan Baugh, Kate Hill-Harfe, Gene Brown and Craig Hunter

Dept. of Molecular and Cellular Biology, Harvard University
Expression Profiling Sciences, Wyeth Research
25 June 2003

Harvard University

Genomic Analysis of Embryonic Gene Expression in *C. elegans*

The nematode *Caenorhabditis elegans*

4-cell-embryo

RNA from FFPE Samples

&

High-Quality Microarrays

BMBF Project “COLOGENETICS”
Euro Trans-Bio
4 partners

Collaboration with AXELA Inc.
Toronto, Canada & Boston, USA
“ZIPLEX” Breast Cancer Prognostic Chip

RNA from FFPE Samples

The Challenge

Limited Sequence Availibility

Cross-Linking: RNA-Protein, RNA-RNA

Problems **Reverse Transcription Stops, difficult to liberate large RNAs**

Chemical Modification: Methylol-Adenines, Dimerized Adenines

Problem **Reverse Transcription Stops, Limited Priming at 3'-Poly(A)**

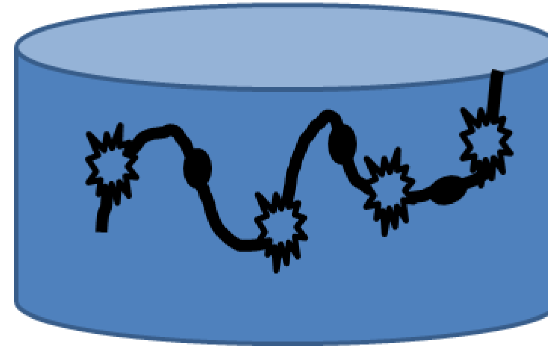
RNA Degradation: Handling, Storage, RNA Isolation

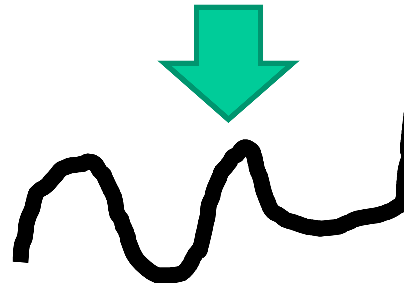
Problem **Many mRNA Fragments without 3'-Poly(A)**

NOW RNA Isolation with FFPE RNAready kit

Experimental Flow Chart

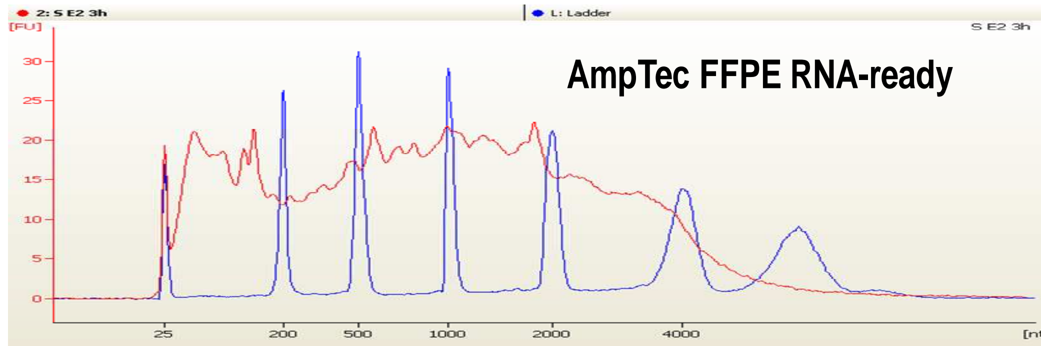
Place up to 5 FFPE sections in a reaction tube
Deparaffinisation Time required: 1 h
Lysis Time required: 3 h
Demodification <u>in Lysis mix</u> Time required: 0.5 h
Spin column purification, including DNA digestion Time required: Approximately 0.75 h
Purified RNA in ~ 50 µl eluate, expected yield: up to 2 µg per slide Total time required: **Approximately 5.5 h**

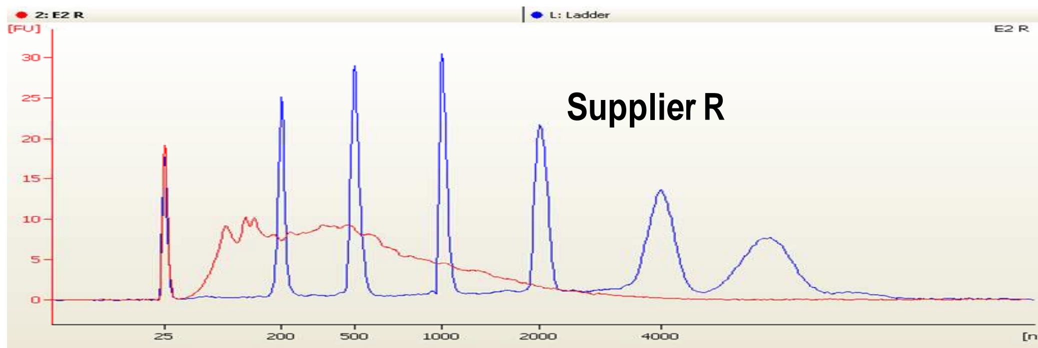


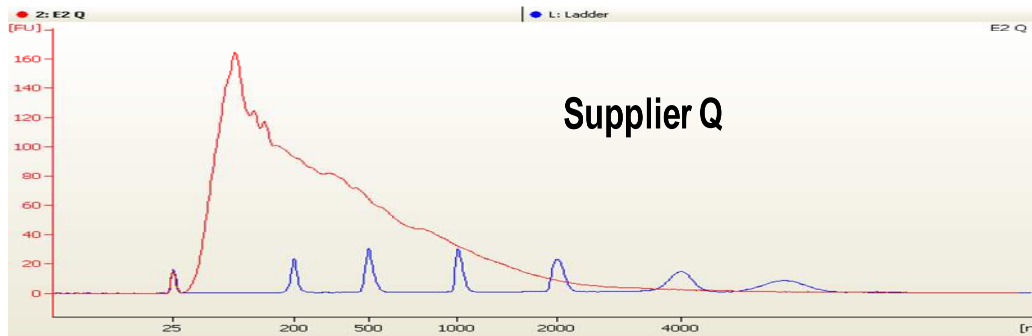


Large RNA in solution

Reduced base modifications







More efficient De-Crossinking: Better liberation of Large RNAs

NEW TECHNOLOGIES FOR FFPE SAMPLES: Improved RNA Isolation and novel cDNA priming for qPCR and for universal mRNA amplification

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Archival FFPE samples have been collected over decades in routine clinical procedures and they harbour a great wealth of information, including mRNA expression profiles. Although the RNA is severely degraded and poses additional challenges due to inter- and intramolecular cross-linking and base modifications. Mining of gene expression data is still possible and extracted information about differential gene expression is comparable to data from Fresh-Frozen samples, even at a quantitative level. Our novel FFPE RNAready kits provide a novel procedure for RNA liberation and demodification, resulting in highly reproducible data in RT-qPCR studies [1] and derived gene expression profiles of cancer samples [2,3] are useful for molecular risk assessments [3].

TR priming/amplification of FFPE RNAs (incorporated in ExpressArt TR mRNA amplification kits) combines advantages of oligo-dT and random priming: **Like oligo-dT**: preferential priming near the 3'end **Like oligo-dT:** selection against rRNAs **Like random**: mRNA fragments without poly(A). **Superior to random**: 3'-preference for full-length "cDNA-fragments", no further "subfragmentation" **Like random:** internal priming for **whole transcript** coverage. **Superior to random**: preferential starts at pause sites

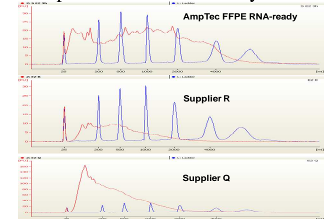
Applications: ◊ qPCR analyses ◊ mRNA amplification and microarray analyses ◊ **Unique advantages for Exon Arrays**

[1] Oberli et al. (2008) Expression profiling with RNA from formalin-fixed, paraffin-embedded material. BMC Medical Genomics 2008, 1:9. / [2] Schobesberger et al. (2008) Gene expression variation between distinct areas of breast cancer measured from paraffin-embedded tissue cores. BMC Cancer 2008, 8:343.
[3] Antonov et al. (201) Molecular risk assessment of BIG 1-98 participants by expression profiling using RNA from archival tissue. BMC Cancer 2010, 10:37

Isolation of FFPE RNA

Size distribution of FFPE RNA

Comparison of commercially available kits



Experimental Flow Chart

Place up to 5 FFPE sections in a reaction tube

Deparaffinisation
Time required: 1 h

Lysis
Time required: 3 h

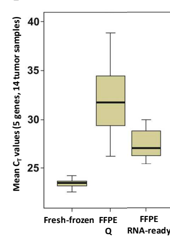
Demodification
Time required: 0.5 h

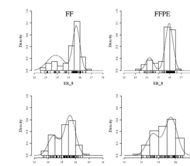
Spin column purification, including DNA digestion
Time required: Approximately 0.75 h

Purified RNA in ~50 µl eluate
Expected yield: up to 2 µg per slide
Total time required: **Approximately 5.5 h**

FFPE RNA as RT-qPCR template

Variability in the qPCR performance of FFPE RNA

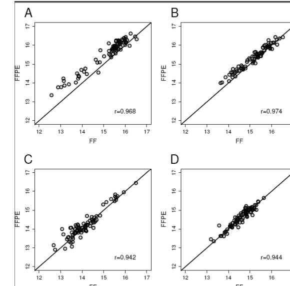




Histograms of ER and PGR scores with fitted mixtures of Gaussian distributions. Fresh-Frozen and FFPE samples result in very similar biphasic distributions.

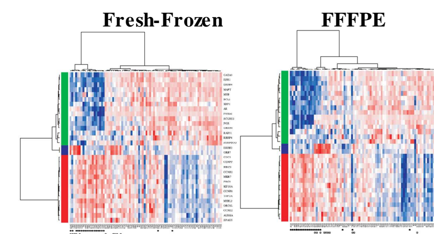
Results of 82 matched samples are shown

Comparison of Molecular Scores derived from Fresh-Frozen vs FFPE tissues



Comparison of scores with intact and FFPE RNA, determined for 82 patients. Scatter plots are shown for scores for each tumor, derived from FF (Fresh-Frozen) and FFPE tissues.

A: ER_8, with 8 genes representing estrog receptor function, **B:** PGR_5 for progesterc receptor, **C:** HER2_2 for Her2 and **D:** PRO_ with 10 genes for proliferation status. Pears correlations are indicated.



Unsupervised hierarchical clustering of data from FF- and FFPE-derived RNA. Shown are heat maps based on normalized expression from RNA of FF and FFPE tissues. Proliferation (red box), Her2 (blue box) and ER or PgR related genes (green box) are indicated.

The hormone receptor status of each tumor was also assessed by IHC. ER negative (black circles) and Her2 positive tumors (open circles) are indicated.

Ziplex Automated Workstation

Samples and reagents are pumped through probe array channels in microplate wells for efficient & **fast** mixing/hybridisation.

Numerical data are available within 3 hours. Automatic Quality Control metrics ensure the output of only high quality data: failure samples with too low signal/background ratios are flagged.

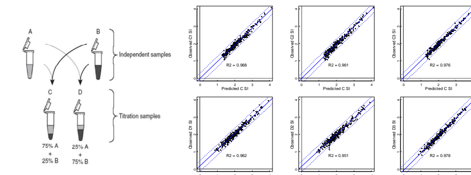
FFPE RNA and Microarrays

Conclusions

- The results demonstrate the feasibility of amplifying and quantifying sequences at any position within transcripts in degraded mRNA from FFPE samples.
- Results accurately reflect transcript abundance in total RNA samples.
- Expression differences of two-fold or less may be analyzed with tens or hundreds of probes for translational research and clinical assay development on the Ziplex Automated Workstation.

"MAQC"- like Sample Titration with FFPE RNAs

Analogous to the MAQC study (Nature Biotechnol. 2006; 24(9):1151-61), FFPE RNAs from breast cancer (sample A) and colorectal cancer (sample B) were mixed to create the C and D titrations. Aliquots of the 4 samples were amplified with the ExpressArt TR kit and hybridised on the Ziplex Workstation. There was good agreement ($R^2 > 0.95$) between the observed and predicted results for C and D samples, calculated from the A and B expression values. Median CV's of the A, B, C and D samples were 19.9, 18.7, 24.3 and 19.6%, respectively.



Observed normalized signal intensities for C and D samples vs. predicted intensities that were calculated from A and B sample data.

Similar performance was observed for 3'-biased probes and for probes several hundred bases away from the 3'end of the mRNA, confirming the lack of 3' bias in the TR amplified RNAs.

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Amplification of Bacterial mRNA *without* rRNA Depletion
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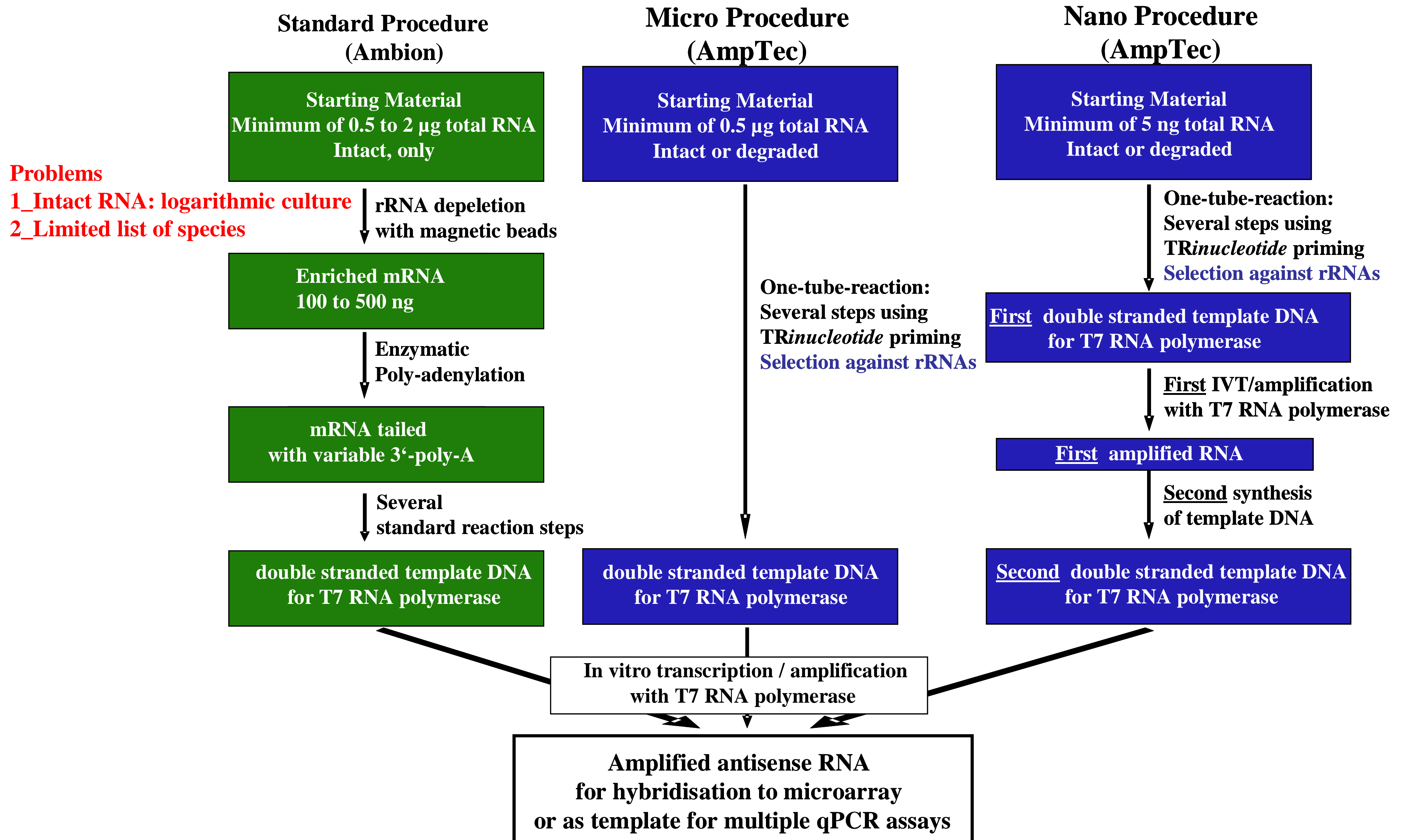

In Vitro Diagnostic Tools

Synthesis of custom-designed, very long RNA or DNA sequences

Applications

At The Start:

Reference RNAs and DNAs for the Development of In Vitro Diagnostic Assays

Continuously:

Internal Control RNA for performance control: RNA isolation, reverse transcription and qPCR amplification/detection

Positive Control RNAs for controlling high assay qualities and specificities

ISO 13485
CERTIFIED FIRM

Key Customers:

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astra diagnostics GmbH, Hamburg, Germany

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