BioSpotlight

Extending MicroRNAs Extend a Hand for In Situ Analysis

It is now clear that microRNAs (miRNAs) are important regulators of gene expression through their repression of mRNA translation, and that they play key roles in development and carcinogenesis. Some studies examining the functions of miRNAs rely upon in situ analysis of their differential expression in developing or cancerous tissues; this is currently done by in situ hybridization using probes that incorporate locked nucleic acids (LNAs), which greatly increase the T_m and specificity of the short oligonucleotide probes necessary to detect miRNAs. In this issue, an alternative in situ technique for tissue sections that should only detect the mature form of an miRNA is described by G. Nuovo, T. Schmittgen, and colleagues at the Ohio State University Medical Center (Columbus, OH, USA). Their method is based upon the in situ hybridization of the mature miRNA to a longer ultramer template, extension of the hybridized miRNA by a polymerase in the presence of digoxigenin- or biotin-labeled reporter nucleotide, followed by detection of the incorporated reporter by

antibody staining. Although a protease and DNase pre-treatment is necessary to eliminate non-specific DNA synthesis on nicked and fragmented genomic DNA in formalin-fixed, paraffin-embedded tissue sections, this method is less expensive than using LNA probes, specific for the mature form of the miRNA, and appears to have a better signal-to-noise ratio.

See “In situ detection of mature microRNAs by labeled extension on ultramer templates” on page 115.

Playing the Slots to Rapidly Assay DNA Damage

DNA damage—such as strand breaks and interstrand crosslinks—induces cellular responses such as changes in replication and the expression of signaling proteins like the double-strand break indicator protein γ -H2AX. These responses are most commonly monitored with flow cytometry for bromodeoxyuridine (BrDU) incorporation or Western blotting, respectively, which are time-consuming or expensive procedures. M. McHugh and T. Beerman of the Roswell Park Cancer Institute (Buffalo, NY, USA) have developed a method for the simultaneous characterization of the type of DNA damage present and either BrDU incorporation or γ -H2AX expression, without the need for cell extraction, using a single membrane in a slot blot. In this assay, cells grown in the presence of ^{14}C -thymidine and a damage-inducing agent (plus BrDU for the replication assay) are added directly to prewetted membranes in a slot blot apparatus. The cells are then lysed and neutralized and the eluted ^{14}C is measured. Because these membranes do not efficiently bind small DNA fragments, ^{14}C -DNA elution from the membrane increases with increasing strand breaks and decreases with crosslinks. The same membranes can then be

stained with chemiluminescent antibody either for BrDU to measure replication (when normalized to the amount of DNA on the blot) or for γ -H2AX. This method should prove to be a useful and rapid first step prior to more rigorous assays of DNA damage and cellular responses.

See “An extraction-free method by which a single slot blot can be used to quantify intracellular DNA damage (crosslinks or strand breaks) and changes in or DNA damage response proteins or replication” on page 127.

Fluoride Protection: for RNA As Well As Teeth

RNA binding proteins (RBPs) are often identified via RNA affinity chromatography, wherein sequences encoding RNA binding elements are immobilized onto a solid matrix, incubated with cellular extracts, and the bound proteins identified by mass spectrometry. RNases present in these extracts are a potential difficulty, since they degrade the RNA probes and inhibit identification of some RBPs. These may be endogenous cellular RNases, or exogenous RNase added in a pretreatment of the extract to eliminate non-probe-specific binding of RBPs that happen to be tethered to the probe-specific RBP through their common binding of

an unrelated RNA. R. Hovhannisyan and R. Carstens at the University of Pennsylvania School of Medicine (Philadelphia, PA, USA) sought to avoid the problem of RNase in cell extracts by synthesizing RNase-resistant probes for use in affinity chromatography. Incorporation of 2'-fluorine-CTP and 2'-fluorine-UTP into the RNAs allowed RNase treatment of cell extracts prior to RNA affinity chromatography, without significant degradation of the immobilized RNA. The fluorine-substituted RNAs coupled successfully to beads via common protocols and it was demonstrated that the protected version of an RNA *cis*-element bound the same protein previously identified with the unmodified probe. This method may be useful in the identification of RNA-protein interactions of readily degradable sequences and verification of direct interactions identified by routine RNA chromatography.

See “Affinity chromatography using 2' fluoro-substituted RNAs for detection of RNA-protein interactions in RNase-rich or RNase-treated extracts” on page 95.

Selected and written by Patrick C.H. Lo, Ph.D. and Kristie Nybo, Ph.D.

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