Alternatives to ^{35}S as a Label for the Differential Display of Eukaryotic Messenger RNA

The differential display of mRNA, as described by P. Liang and A. B. Pardee (*1*), is an important tool to detect and characterize altered gene expression in eukaryotic cells. This comment addresses a problem of using α-[^{35}S]2′-deoxyadenosine 5′-triphosphate (α-[^{35}S]dATP) as a label in polymerase chain reaction (PCR)–based procedures. It is known that volatile ^{35}S-labeled decomposition products may form during storage or use of α-[^{35}S]dATP. Therefore, manufacturers recommend to conduct operations using α-[^{35}S]dATP in a vented fume hood. Although such decomposition is minor at room temperature, we found α-[^{35}S]dATP to form high levels of volatile ^{35}S-labeled decomposition products during the PCR reaction used in differential display. The high temperature used in thermal cyclers increased the rate of decomposition significantly, leading to a high contamination of the PCR machine. This became evident because the thermal cycler used (PTC-100-60, MJ Research, Watertown, Massachusetts) is equipped with a heated lid that prevented, at least partially, the escape of volatilized radioactivity. Several brands of PCR tubes were tested, and the PCR reaction mixture was kept under 100 μl of paraffin oil. However, the radioactive decomposition products diffused through the cap under all conditions tested. For this reason, we recommend the use of ^{32}P or ^{33}P instead of ^{35}S as a label for the differential display of mRNA.

^{32}P is a suitable alternative to label PCR products (Fig. 1). We followed the protocol of Liang *et al.* (*2*) (except for the use of only one 3′ primer per reaction and for the labeling procedure) for the differential display of mRNA to identify ethylene-regulated genes from 4-day-old etiolated seedlings of *Arabidopsis thaliana*. Although the use of α-[^{35}S]dATP as a label gave a clear pattern of differentially expressed bands (Fig. 1A), the use of ^{35}P yielded the same pattern of ethylene-regulated genes (Fig. 1, B, C, and D). For labeling, ^{32}P end-labeled oligo(dT) primer or α-[^{32}P]2′-deoxycytidine 5′-triphosphate (α-[^{32}P]dCTP) was used in the PCR reaction. The end-labeling of the oligo(dT) primer gave a stronger signal with smaller PCR products (Fig. 1B), whereas the use of α-[^{32}P]dCTP in the PCR reaction favored more intense labeling of larger DNA fragments (Fig. 1C). In order not to miss any differentially displayed bands, two different lengths of exposure were required, or end-labeled primers were used together with free α-[^{32}P]dCTP in the same reaction mixture (Fig. 1D).

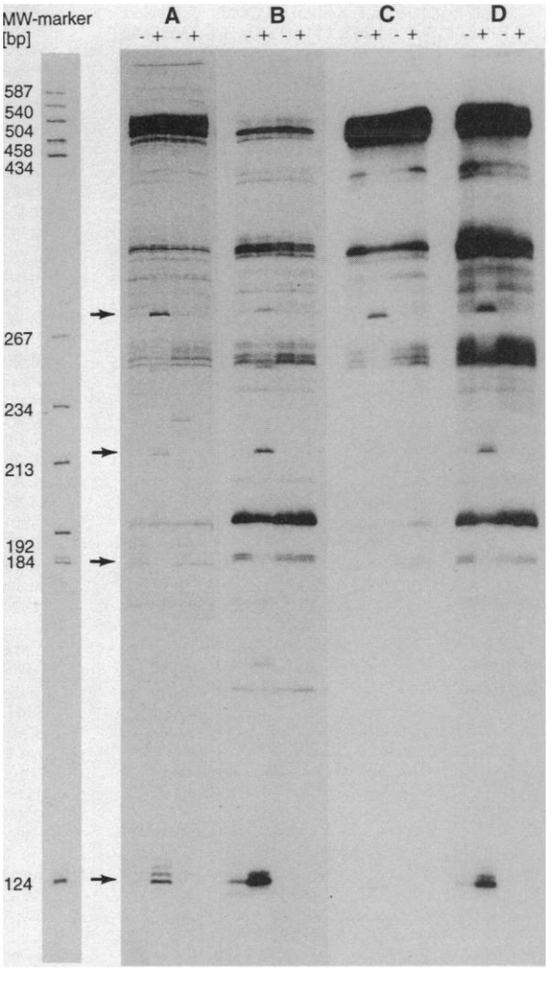

Fig. 1. Comparison of different methods of labeling for differential display of eukaryotic mRNA. Left lane shows the ^{32}P end-labeled DNA molecular weight markers V (Boehringer Mannheim, Indianapolis, Indiana). RNA for the differential display was isolated from 4-day-old etiolated seedlings of *Arabidopsis thaliana* wild type (WT) and the ethylene-insensitive mutant *etr* (ETR) (*3*) grown for 4 days under air (−) or exposed to ethylene (5 μl/liter) for 6 hours before harvesting (+). The arrows show three mRNAs induced and one mRNA repressed by ethylene. Panels represent different methods of labeling: (**A**) 1 μl of α-[^{35}S]dATP (1200 Ci/mmol) was used in the PCR reaction; (**B**) one-fourth of the oligo(dT) primer was end-labeled using 2 μCi of γ-[^{32}P]ATP (6000 Ci/mmol) per reaction; (**C**) 1.5 μCi of α-[^{32}P]dCTP (3000 Ci/mmol) was used in each PCR reaction; (**D**) the oligo(dT) primer was end-labeled as in (B), and an additional 1 μCi of α-[^{32}P]dCTP was added to each PCR reaction. All isotopes were purchased from New England Nuclear (Boston, Massachusetts).

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Response: Trentmann *et al.* raise a potentially serious problem with regard to the use of α-[^{35}S]dATP as a label for differential display. Their observation of ^{35}S contamination of the thermocyclers during PCR amplification has been confirmed in our laboratory. The decomposition of [^{35}S]dATP into a volatile product during PCR and escape of the radioactivity from the tops of the PCR reaction tubes could be hazardous to researchers. We appreciate the opportunity to bring this safety issue to researchers who are practicing differential display.

The choice of which isotope to use for differential display is determined by several factors, including the sensitivity of detection, band resolution, safety, and cost. The isotope α-[^{35}S]dATP was originally described for differential display (*1*, *2*) for the purpose of obtaining high resolution of bands, as it is widely used for DNA sequencing. The isotope is also convenient because minimal physical protection is needed given the large number of PCR reactions and gels required for the procedure. However, ^{35}S contamination of the thermocyclers raises the question of whether α-[^{35}S]-dATP should be continuously used for the method, especially if ^{35}S could escape from the tops of the PCR tubes. We first noticed

^{35}S contamination of our thermocycler when thin-walled tubes were used for the PCR reaction. The contamination could not be completely cleaned away. One possible solution, if ^{35}S has to be continuously used for differential display, is to have the contaminated thermocyclers dedicated for the work, and we recommend that thermocyclers without heated lids be kept under a hood to prevent release of the isotope through the tops of the PCR tubes.

Trentmann *et al.* suggest that the use of α-[^{32}P]dCTP instead of α-[^{35}S]dATP in differential display should reduce the risk of isotope contamination and also increase the sensitivity of the method. ^{32}P was used successfully before for RAP-PCR, a method similar to differential display (*4*). But the drawbacks for the routine use of [^{32}P]-dCTP are that physical protection (a shield) is required during the experiment, and the band resolution is low, as a result of the much stronger radiation of ^{32}P. This may pose a technical difficulty, especially when large numbers of PCR reactions are to be prepared and thousands of bands are to be resolved. Fortunately, recent commercial availability of ^{33}P-labeled nucleotides (which has a radiation energy and half-life between that of ^{32}P and ^{35}S) seems to provide a solution that combines the advantages of both ^{35}S and ^{32}P labels. We have recently demonstrated that the use, per PCR reaction, of 1 μCi of α-[^{33}P]-dATP (NEN) instead of 5 μCi of α-[^{35}S]-dATP gave superior sensitivity and excellent band resolution (*5*) (data not shown). The results indicate that α-[^{33}P]dATP gives higher sensitivity (more intense bands) and an otherwise similar pattern of cDNAs displayed. (Therefore, the cost factor for α-[^{33}P]dATP becomes less of an issue.)

Trentmann *et al.* also compare the use of direct incorporation of α-[^{32}P]dCTP and ^{32}P end-labeled primers for differential display and observed that the latter favors the detection of the smaller molecular bands. This is consistent with the fact that PCR favors the amplification of the smaller DNA products, and each product amplified will contain only one labeled primer, in comparison with the direct incorporation method, in which the greater the lengths of the products, the more labeled nucleotides will be incorporated. This may explain why higher molecular weight bands are underrepresented when labeled primers are used. The combinational use of both labeled primers and direct incorporation of labeled nucleotides may allow more bands to be detected. However, the shelf-life of labeled primers and batch-to-batch consistency of primer labeling efficiency may pose problems of technical difficulty and reproducibility of the method.

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