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Detection of UV-induced RNA:protein crosslinks in snRNPs by oligonucleotides complementary to the snRNA

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Covalent crosslinking of proteins to RNA followed by analysis on SDS–polyacrylamide gels is commonly used as a method to characterise ribonucleoprotein complexes (1–3). However this method usually requires either extensive purification of the complex and labelling of the RNA, detection of the RNA by hybridisation after blotting, or the ability to form the complex *in vitro* with radioactively labelled RNA. We show here that these problems can be overcome if the RNA in the crosslinked products is detected by hybridisation with a short radioactively labelled complementary oligonucleotide. Such hybrids can be sufficiently stable to survive SDS–polyacrylamide gel electrophoresis and the method has the additional advantage that the decorating oligonucleotide only moderately affects the mobility of the detected molecules. Similar 'complementary oligonucleotide decoration' methods were previously introduced for the rapid indirect end-labelling and restriction mapping of DNA cloned in lambda phage vectors (4) or for the characterisation of snRNPs by non-denaturing gel electrophoresis (5, 6).

We have used this method to characterise close RNA–protein contacts within the high-abundance U1 ($\sim 10^6$/cell) and low-abundance U7 snRNPs ($2-20\times10^3$/cell; reviewed in 7). Both U snRNAs contain single-stranded regions at their 5′ ends available for base-pairing with substrate RNAs. Moreover, both snRNPs contain a full set of the common snRNP proteins (Sm proteins) and additional snRNP-specific proteins [70 k, A, and C for U1 and proteins of 50 and 14 kD for U7; (8, 9)]. For U1, the common 9-kD G protein has been shown to become crosslinked to the RNA after UV irradiation (3).

UV crosslinking was carried out at room temperature in Eppendorf tube caps. 5 μl of nuclear extract from K21 mouse mastocytoma cells (10) containing 10 mM EDTA and 50 ng of denatured hering sperm DNA (as unspecific competitor) was irradiated for 30 min under the 254 nm lamps of a Hanau Fluotest Universal illuminator. Then, 25,000 cpm of an oligodeoxynucleotide complementary to either nt 1–16 of U7 RNA [oligo cA; (6, 11)] or nt 10–25 of U1 RNA (oligo U1a), 5′-endlabelled with γ^{32}P-ATP and T4 polynucleotide kinase, was added and incubation continued at 30°C for 30 min. Heparin was added to 5 mg/ml and incubation continued at room temperature for 10 min. After addition of SDS loading buffer (50 mM Tris HCl, pH 6.8, 10% glycerol, 100 mM DTT and 2% SDS, 0.1% bromophenol blue), the samples were loaded without boiling onto a 10% discontinuous SDS–polyacrylamide gel (12). After electrophoresis, the gel was autoradiographed without drying and marker lanes were stained with Coomassie blue.

Without UV-crosslinking, the analysis by SDS–PAGE allows one to detect U7 (Figure 1, lane 1) or U1 RNA (lane 3) efficiently and specifically. U7 RNA decorated by oligo cA migrates with an apparent molecular weight (MW) of about 21 kD (compared to the theoretical value of 26 kD), slightly above the broad band caused by free oligo cA. U7 RNA detection is lost if the extract is digested with micrococcal nuclease, but is retained in RNA samples prepared from nuclear extract (data not shown). The intensity of the U7-specific band is reduced if the samples are boiled prior to electrophoresis, but hybrids can reform if the samples are kept at room temperature after boiling. Formation of the U7-specific band can be competed by unlabelled oligo cA, but not by other oligonucleotides. The U1-specific band has an apparent MW of 55 kD (theoretically 60 kD). Several fainter bands of lower MW that are also decorated by oligo U1a presumably represent degradation products. Detection of U1 RNA is less efficient than for U7, especially considering the large difference in abundance of these snRNPs. Most likely this is because the U1 sequence complementary to the oligo is partly contained in an RNA secondary structure (7).

When the nuclear extract is irradiated with UV light prior to incubation with the oligo and SDS–PAGE, two additional bands are obtained for U7 (lane 2) with apparent MW of 35 and 70 kD, respectively. These bands, like the lower band corresponding to free U7 RNA, are strongly reduced in intensity if the samples are boiled prior to electrophoresis and show the same sensitivity to competitors (data not shown). For U1 RNA, a single crosslinked product with apparent MW of 65 kD is observed (lane 3).

To estimate the size of these proteins, one has to subtract the apparent MW of the band corresponding to the free snRNA. This results in MW of 14 and 50 kD for the U7-specific crosslinks and of 10 kD for the U1-specific crosslink, respectively. Since UV-crosslinking is performed on the extracts as they are, i.e. without addition of any further reagents, and since the covalent crosslinking by UV light requires the reacting groups to lie within

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atomic distance (1), it appears that proteins of these sizes are in close contact with U7 and U1 RNA within the respective snRNP particles.

Although these size estimations, for various reasons, are not very precise, the protein giving rise to the upper U7-specific product is most likely identical to a recently identified 50-kD U7-specific protein (9). The identity of the smaller protein is less clear. A 14-kD U7-specific protein has been identified (9), but several of the common Sm proteins also lie within the size range of 9–16 kD (8). In the light of the recent UV-crosslinking study by Heinrichs *et al.* (3) the U1-specific crosslink is almost certainly due to the 9-kD G protein. After recovery of the crosslinked complexes from the gel, it may be possible to identify individual proteins or to map the site of crosslinking by immunoprecipitation and primer extension analyses, respectively. Furthermore, the method should be equally applicable to 2′-O-methyl or 2′-O-allyl oligonucleotides.

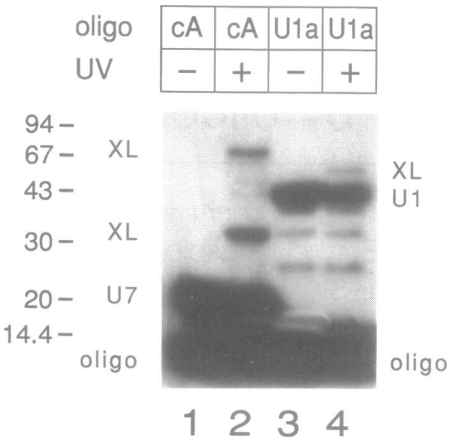

Figure 1. UV-crosslinking of specific polypeptides to U7 (lanes 1, 2) or U1 RNA (lanes 3, 4) in nuclear extract from K21 mouse mastocytoma cells. Nuclear extract was irradiated with 254 nm UV light (lanes 2 and 4) or mock-treated (lanes 1 and 3), incubated with radiolabelled oligo cA complementary to nt 1–16 of U7 RNA (11; lanes I and 2) or Ula complementary to nt 10–25 of Ul RNA (7), and then subjected to SDS–polyacrylamide gel electrophoresis (12). The positions of size markers are indicated on the left.

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