HYBRIDOCYTOCHEMISTRY WITH 2-ACETYLAMINOFLUORENE-MODIFIED PROBES

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The detection of specific nucleic acid sequences in microscopic preparations can be achieved by hybridocytochemical procedures. For this purpose, the DNA in microscopic preparations is fixed and dentured, and incubated with the labeled nucleic acid probe under hybridization conditions.

Our group has developed several methods which replace radioactive markers by labels like fluorochromes and cytochemically detectable enzymes. These procedures have as main advantages speed and localization precision compared to autoradiography.

Various approaches have been developed to provide the specific hybrids with these labels. They are either introduced directly to the probe, by coupling a fluorochrome covalently to the 3'-terminus of RNA [Bauman et al., Exp.Cell Res. 128 (1980) 485], or in an indirect manner by means of specific antibodies to which these labels are bound.

For the indirect approaches an element in the hybrid has to act as an antigen. This could be the hybridized sequences themselves as is the case in the method of Van Prooijen-Knegt et al. [Exp.Cell Res. 141 (1982) 397], where the generated RNA.DNA hybrids are immunologically distinct from DNA.DNA- and RNA.RAN duplexes, or a hapten, artificially introduced into the probe.

Here we present a novel method in which the probe is rendered immunogenic by chemical attachment of 2-acetylaminofluorenyl (AAF) groups. After hybridization, the labeled probes are recognized by antibodies directed against AAF-modified guanosine and visualized immunocytochemically [Landegent et al., submitted].

Some specifications of this procedure are:

1) The modification procedure is relatively easy and fast. Covalent binding of AAF-groups is achieved by treatment of the polynucleotide sequences with N-acetoxy-2-acetylaminofluorene (N-AcO-AAF), a reactive derivative of the well-known and widely studied chemical carcinogen [Kriek et al., Biochemistry 6 (1967) 177]. The reaction itself is complete within 20 to 30 min of incubation.

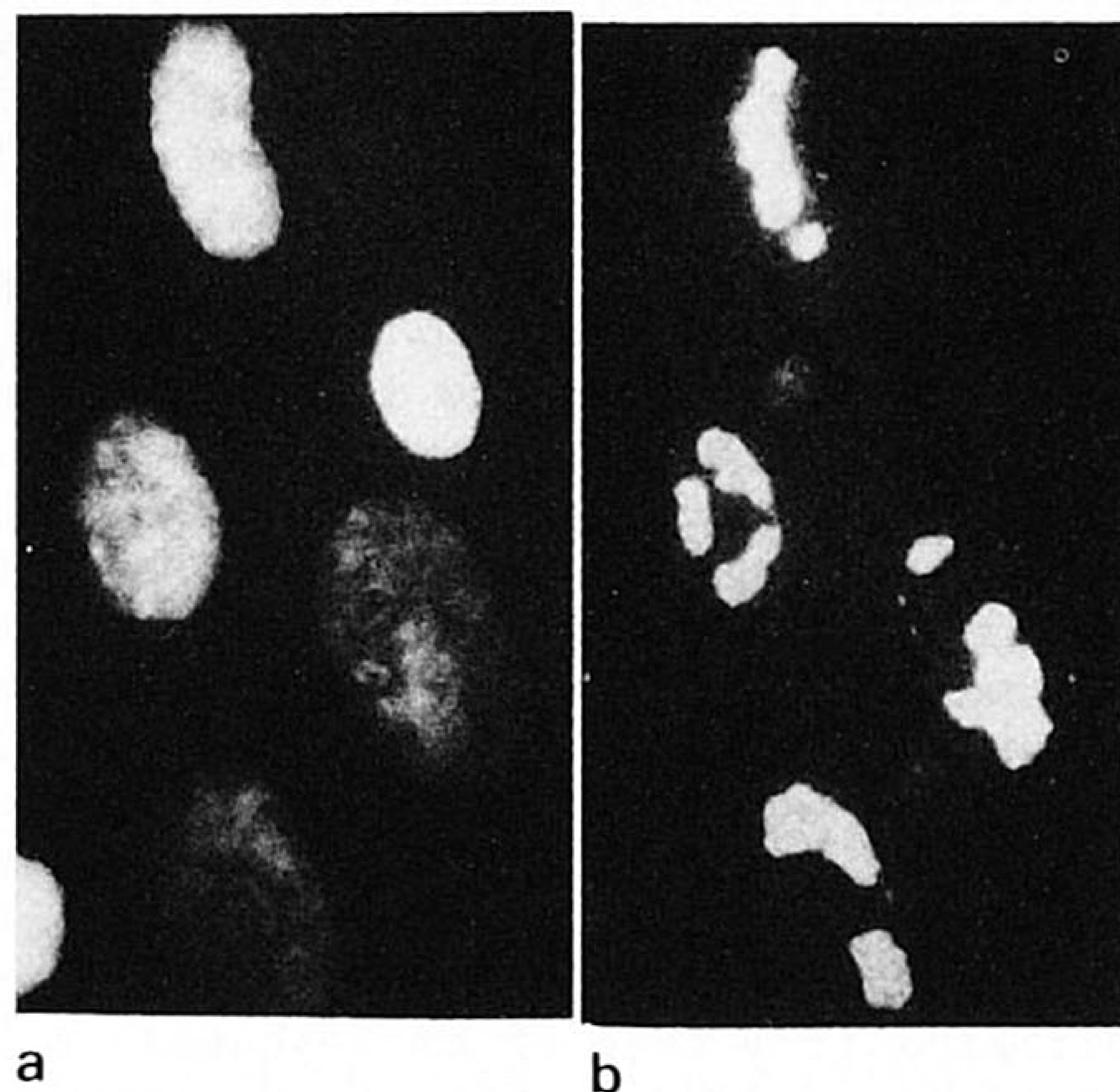

Fig. 1: Hybridization of human cytomegalovirus cRNA, modified with N-AcO-AAF to a level of about 5%, to a preparation of virus-infected human embryonic lung fibroblasts. With the DAPI fluorescence (general DNA stain), the nuclei are all visible (a). Some of them are labeled specifically with TRITC (b). Which means that these cells contain CMV-sequences.

2) The labeling is reproducible. Our experiments indicated that the number of bound carcinogen is proportional to the initial concentration of N-AcO-AAF added. The exact extent of AAF-modification can be easily determined optically from the A305/A260 ratio, or by scintillation couting after addition of a trace amount of N-AcO-[3H]-AAF.

3) Both double stranded and single stranded probes (DNA as well as RNA) can be modified with the same efficiency because N-AcO-AAF mainly reacts with the C-8 position of guanin yielding N-(guanin-8-yl)-AAF adducts.

4) The AAF-adducts are strong immunogens. Antisera with high affinity constants $>10^9$ mol^{-1} have been elicited by several groups.

The feasibility of this new approach is demonstrated by the localization of several specific sequences, such as satellite DNA and 18S- and 28S rRNA in metaphase chromosomes, and viral sequences in infected cells.

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