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Immunogold cytochemistry in plant virus research. A review.

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Abstract. The technique of using antibodies or protein A labelled with colloidal gold for the detection of antigens at the ultrastructural level has only recently come into use in plant virus research. The reports published to date are reviewed and some further possibilities for the use of immunogold techniques in plant virus research are discussed.

Index words: immunogold cytochemistry, plant viruses

Introduction

Over the years many investigators have attempted to determine the subcellular sites of virus replication and the sites of synthesis of virus coded proteins in infected plant cells. Plant cells present many problems as compared to animal cells because of the limited permeability of the cell wall and the considerable asynchrony of infection in intact plant tissue.

The immuno-ferritin technique has been used to localize plant viral protein with pre-embedding staining (SHALLA and AMICI 1967, SHEPARD et al. 1974, HATTA and MATTHEWS 1976). However, the relatively large ferritin-antibody complex penetrates poorly and binds non-specifically. Under the conditions necessary for the diffusion of the labelled antibodies redistribution of viral protein cannot be excluded.

In recent years techniques have been developed using immunological reagents labelled with colloidal gold. These can be used on ultrathin sections and overcome many of the problems with pre-embedding staining. The immunogold technique has been used in plant virus research only during the last four years. The reports that have appeared to date are briefly reviewed here and the potential of immunogold techniques in plant virus research is discussed.

Methodology

The basic principle is that thin sections are treated with antibodies specific for the anti-

gen to be localized. These antibodies can be labelled with colloidal gold, but more commonly a two-step procedure is used in which the specific antibodies are detected by a labelled second antibody (LIN and LANGENBERG 1983) or labelled protein A (ROTH et al. 1978). The gold particles are easily seen in the electron micrographs.

Tissue to be used for immunogold studies is fixed in glutaraldehyde. Post-fixation in OsO_4 is usually omitted since it considerably reduces antigenicity (LIN and LANGENBERG 1983). Various embedding media have been tried: Epon (GARNIER et al. 1986), Araldite, Lowicryl, and LR Gold (LIN and LANGENBERG 1983, TOMENIUS and CLAPHAM 1985 a, b, LANGENBERG 1986). Sections of embeddings in Epon or Araldite are usually etched with hydrogen peroxide (GARNIER et al. 1986) or alcoholic sodium hydroxide (LIN and LANGENBERG 1983) in order to improve accessibility of antigenic sites. Such treatment has adverse effects on ultrastructure (TOMENIUS and CLAPHAM 1985a). Etching can be dispensed with when Lowicryl or LR Gold is used and these embedding media are therefore preferred (LANGENBERG 1985, 1986, TOMENIUS et al. 1987).

The sections are mounted on Formvar coated nickel grids. They are first treated with a 1 % solution of bovine serum albumin to reduce non-specific adsorption of immunoglobulins and then incubated with the specific antibody. After washing in buffer they are incubated with the gold-labelled second antibody or protein A followed by a final wash. The grids are kept floating on, or immersed in, drops of the appropriate solution and placed on a magnetic stirrer to ensure gentle movement during all incubation and washing steps. The sections can then be stained with uranyl acetate and lead citrate (LANGENBERG 1985, TOMENIUS et al. 1987).

Applications

The first reports on the use of immunogold cytochemistry in the study of plant viruses appeared in 1983. LIN and LANGENBERG (1983) used the technique to localize barley stripe mosaic virus in infected wheat cells. After applying the gold-IgG complex they found strong uniform specific labelling over crystalline aggregates of virus particles. A quantitative evaluation of the specificity was made. Although the labelling was not very intense — on the average 1.5 gold particles per virus rod — there was a linear relationship between gold particles and virus rods. The low level of labelling is explained by the fact that only antigenic sites on the surface of the sections are accessible, the resin being impermeable to the antibodies.

TOMENIUS et al. (1983) used immunogold cytochemistry to localize red clover mottle virus (RCMV) antigen in infected pea leaf cells. Label was intense in the cytoplasm and among the membranes of the virus-induced membranous inclusions but not over the vesicles. Due to the relatively poor contrast in tissue fixed with glutaraldehyde alone virus particles were generally not seen. However, since gel diffusion tests showed that the antiserum used was specific for whole virus and did not react with coat protein subunits it was concluded that the gold label showed the location of virus particles.

In further studies (TOMENIUS et al. 1984) using antibodies specific for the larger (40K) capsid protein of RCMV it was shown that label was generally distributed over the cytoplasm and among the membranes of the virus-induced inclusions. The capsid protein was detectable simultaneously with intact virus but could not be detected before the membraneous inclusion had developed. There was no indication of accumulation of the capsid protein at any specific site or in any organelle. Antibodies specific for the smaller (22K) capsid protein have also been produced and they are being used in studies with the immunogold technique (unpublished).

LANGENBERG (1985) studied wheat doubly infected with wheat spindle streak mosaic virus (WSSMV) and soil-borne wheat mosaic virus (SBWMV). He found that SBWMV

antibodies reacted with virions and inclusion bodies of both viruses in thin sections, although when tested with virions in leaf dip preparations, the two antisera were completely specific. The conclusion was that SBWMV capsid protein or inclusion body protein apparently binds to WSSMV pinwheels and virions aggregates *in vivo* in doubly infected cells.

LANGENBERG (1986) demonstrated the presence of capsid protein or virions in cylindrical inclusions induced by two viruses that infect wheat, WSSMV and wheat streak mosaic virus. He hypothesized that the cylindrical inclusions play a role in the cell-to-cell movement of virions through the plasmodesmata.

LANGENBERG and VAN DER WAL (1986) could indentify barley yellow mosaic virus in roots of infected barley by immunogold labelling. They also stained sections containing various stages of *Polymyxa graminis* which is known to be a vector of BYMV and *Lagena radicicola* which is a possible vector. In no case did they find label above background.

In recent years a number of virus-coded proteins other than the structural proteins have been identified and antibodies to them have been produced. It is thus possible to study such proteins with the immunogold technique. GIBAND et al. (1984) used immunogold cytochemistry for the in situ localization of cauliflower mosaic virus and the major protein of the virus-induced inclusion bodies.

GARNIER et al. (1986) studied cells infected with turnip yellow mosaic virus. They used antibodies to the RNA replicase and its 115K virus-coded subunit. Label was found at the periphery of chloroplasts, which confirmed earlier findings where the replicase was localized by its synthetic activity. However, they also found label in the cytoplasm and concluded that this probably represented newly synthesized replicase or its 115K subunit.

TOMENIUS et al. (1987) used immunogold cytochemistry to localize the virus-coded 30K protein in tobacco mosaic virus infected leaves. This protein has been proposed to have a function in the cell-to-cell movement of the virus. The 30K protein was localized to the plasmodesmata. There was an accumulation inside the plasmodesmata with a maximum about 24 hours after inoculation. No specific label was found in the nucleus or at any other site in the cells.

Although single-stranded nucleic acids are not immunogenic, double-stranded nucleic acids are. LIN and LANGENBERG (1985) used a poly(I):poly(C) antiserum to study root tips of wheat systemically infected with barley stripe mosaic virus. They found gold label in vesicles in proplastids thus supporting the notion that proplastids play a role in BSMV replication.

Work in progress in this laboratory (unpublished) using a poly(A):poly(U) monoclonal antibody (DELAGE et al. 1984) has shown that double-stranded RNA can be localized in plant cells infected with TMV and RCMV, respectively.

In addition to applications in cytochemistry, gold-labelled antibodies can be used for enhancement of decoration of virus particles in immunosorbent electron microscopy (LANGENBERG 1985, TOMENIUS and GIDLUND 1986). This should prove useful in particular with small spherical viruses present in low concentration.

Discussion

The immunogold technique, although it still has its limitations, has a considerable potential in plant virus research. A limitation is that tissue prepared without OsO_4 fixation has relatively poor contrast: membranes are not visible and small spherical virus particles are difficult to see unless present in crystalline aggregates. Attempts have been made to overcome these difficulties (TOMENIUS and CLAPHAM 1985 a, b,) but it is still necessary to compromise between good contrast and well preserved ultrastructure on the one hand, and retained antigenicity on the other.

As pointed out earlier only antigen at the very surface of the section can be labelled. However, as shown in several of the reports

reviewed here, the specificity is good, and even though the label is not intense, it is still sufficient for a quantitative evaluation.

By careful adjustment of the conditions in the preparation of colloidal gold it is possible to obtain gold particles of defined size (FRENS 1973). This opens the possibility to do double labelling experiments. Antibodies specific for two different antigens, e.g. two virus-coded proteins can be applied and in the second step detected by second antibodies labelled with gold particles of different sizes. Work in progress in this laboratory (unpublished) with antibodies to RCMV and the two capsid proteins has shown that this is indeed possible.

A possibility that has yet to be exploited is to use cDNA probes for the detection of viral nucleic acids on thin sections. cDNA can be biotinylated and detected by antibodies to biotin; this technique is used in filter hybridization tests for the detection of viruses and viral nucleic acids. In principle, it should be possible to use it in combination with immunogold cytochemistry.

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SELOSTUS

Kultaleimaus kasvivirusten immunoelektronimikroskopiassa

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Vasta-aineiden tai proteiini A:n kultaleimaukseen perustuvilla immunoelektronimikroskooppisilla menetelmillä tutkitaan antigeenejä hienorakennetasolla. Vasta viime vuosina tätä menetelmää on kokeiltu myös kasviviruksiin. Katsauksessa esitetään menetelmän perusteet ja tarkastellaan sen tärkeimpiä sovellutuksia kasvivirusten tutkimuksessa.

Kultaleimauksella on tutkittu mm. virusten sijaintia kasvin juurissa ja lehdissä. Sillä on paikannettu virusten tuottamia proteiineja ja selvitetty niiden merkitystä.

Menetelmän heikkoutena on toistaiseksi se, että ilman osmiumtetroksidivärjäystä kasvisolut antavat elektronimikroskoopissa heikon kontrastin, jolloin tietyt solun osat ja erityisesti eräät viruspartikkelit erottuvat vain kasaumina. Lisäksi vain aivan leikkeen pinnalla olevat antigeenit voidaan leimata. Rajoituksista huolimatta kultaleimaustekniikalla on laajat sovellutusmahdollisuudet kasvien virusinfektioiden perusmekanismien tutkimisessa.