

Expression screening for interacting proteins using immunochemical detection

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Screening of cDNA expression libraries is a well established and frequently used method for cDNA cloning. Generally, cDNAs are inserted in expression vectors like λ gt11 or λ ZAP as fusions with the lacZ gene such that they are under the control of the lac promoter. After plating recombinant bacteriophages on an appropriate bacterial strain, expression of fusion proteins is induced (1). Libraries are usually screened either with an antibody directed against the protein of interest or by use of protein-specific ligands (1). Recently, binding partners for known proteins were identified by screening with the respective radiolabeled protein (2,3). However, the necessity to radiolabel the probe may be a drawback of this method as the respective protein needs to be highly purified before it can be iodinated or possibly phosphorylated *in vitro*. Similarly, *in vivo* labelling will require subsequent purification. In order to circumvent these difficulties, we devised a non-radioactive immunochemical technique for screening expression libraries by use of a protein probe. We also used covalent cross-linking for stabilizing protein-protein interactions so that quite stringent screening conditions can be employed. Clones expressing the target protein were detected by immunostaining with an antibody directed against the probing protein. This method allowed us to isolate the cDNA coding for a novel protein that binds to the glucocorticoid receptor.

For probing we here used the mouse glucocorticoid receptor produced in the Baculovirus expression system (4). Cytosolic extracts of infected insect cells were treated with high salt for receptor activation (5) and aliquots containing 3–6 μ g receptor (as judged from hormone binding capacity) were used per nitrocellulose filter. A λ gt11 human liver cDNA library (Clontech, Palo Alto, USA) was plated (10^4 pfu per 90 mm Petri dish), induced, and transferred to nitrocellulose (1). Filters were washed with saline and blocked for 1 h at 8° in 20 mM Hepes, pH 7.4, 10% glycerol, 5% skimmed milk powder, 0.1% NP-40, 50 mM KCl, 10 mM MgCl₂, 1 mM DTT. Incubation with the receptor probe was over night at 8° in 5 ml of the above solution except that the milk protein concentration was reduced 5-fold. After briefly rinsing with buffer the filters were exposed for 1 h in the cold to 14 mM dimethyl suberimidate (Pierce, Rockford, IL, USA) for chemical cross-linking (6). Filters were washed extensively at room temperature with saline containing 0.3% Tween 20. Positive plaques were visualized by immunostaining with the receptor specific monoclonal antibody mab49 (7) and a peroxidase conjugated second antibody (8).

When we screened 6×10^5 pfu of the λ gt11 library by this method we found a single positive clone (Fig. 1A). It contained a cDNA insert coding for the major part of a novel protein. Expression as a fusion protein in *E.coli* resulted in a product which specifically interacts with the glucocorticoid receptor *in vitro*.

In order not to re-isolate the cDNA for the human receptor we used in our screening experiments the mouse rather than the human receptor protein since the applied monoclonal antibody does not cross-react with the human protein (9). However, if no suitable antibody is available which distinguishes between species, false positive plaques resulting from cross reactivity may easily be identified by screening replica filters with the antibody itself in control experiments similar to that of Fig. 1D. A comparison of the filters presented in Fig. 1C and D in fact shows the specificity of the reaction with the receptor protein.

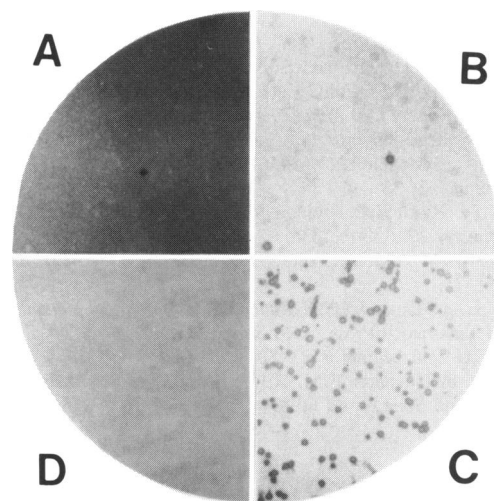


Figure 1. Detection of positive phage clones. Segment A shows the primary positive clone detected by indirect immunostaining as described in the text. This clone was purified over 2 rounds of plating (B and C). Segment D shows a control screen from which the receptor protein was deleted. Peroxidase staining was with 3,3'-diaminobenzidine in the presence of NiCl₂ (8).

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Interestingly, phage plaques not expressing the receptor associating protein may show up as phantom images (Fig. 1B) again stressing the significance of the protein-protein interaction.

In the experiments described here we used covalent cross-linking for stabilizing protein-protein interactions. This allows stringent washing of the nitrocellulose filters and certainly minimizes the danger of disrupting relevant protein-protein interactions during subsequent handling. However, chemical cross-linking may not be required depending on the individual experimental system. The efficiency of cross-linking also depends on the availability of the reactive groups on either protein, their steric juxtaposition, as well as the cross-linking span of the bifunctional reagent used (10). Rational predictions with respect to these parameters are usually not possible.

The method described here will be of general use for studies of protein-protein interactions. Major advantages are that no radioisotopes are required and that there is no need to purify the protein used for screening as specificity is provided by an antibody. Furthermore, the probing protein utilized in this procedure is in a functionally intact state as it is either obtained directly from mammalian cells or overexpressed in the Baculovirus system. Compared to the yeast two-hybrid screening system (2,11) our protocol is very easy to use and it is less susceptible to experimental problems.

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