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Inverse polymerase chain reaction for rapid gene isolation in *Arabidopsis thaliana* insertion mutants.

Recently, many mutants have been isolated in the model plant *Arabidopsis thaliana* by the insertion of the *Agrobacterium tumefaciens* T-DNA into the plant genome (KONCZ *et al.*, 1990; FELDMANN *et al.*, 1991; VAN LIJSEBETTENS *et al.*, 1991).

Instead of applying Southern analysis on these insertion mutants and to avoid the construction of mutant-derived genomic libraries, we propose a much shorter and less laborious strategy for cloning the wild-type genes corresponding to the mutant loci by making use of the polymerase chain reaction (PCR) technology. For the analysis, we used the transgenic line *pfl*, bearing a mutation that alters leaf morphology, induced by a T-DNA insertion (VAN LIJSEBETTENS *et al.*, 1991).

The intactness of the right T-DNA border was checked in a direct PCR making use of 10 ng total genomic plant DNA in a 50-μl reaction mixture containing buffer (1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.2 mM gelatin), 0.2 mM of each dNPTs, 0.2 μM of each primer, and 2.5 units of *Taq* polymerase. Several sets of 25-base-pair (bp) primers with 50 to 60 % GC content were chosen that revealed amplified fragments between 500 and 1000 bp after 30 cycles (20 seconds at 94°C, 20 seconds at 53°C, and 60 seconds at 72°C) indicating that the right T-DNA border was intact.

Then, plant DNA flanking the right T-DNA border was amplified by using inverse PCR (OCHMAN *et al.*, 1988). Primers with homology to the right T-DNA border were chosen in opposite direction. In this reaction, the genomic plant DNA was digested and ligated in conditions that favor the formation of a pool of monomeric circles, one of which served as template in the inverse PCR (IPCR). The same reaction conditions as for direct PCR were used.

We determined that 10 ng of total ligated plant DNA was sufficient for IPCR amplification of a *Sau*3A- or *Dra*I-generated DNA fragment of 800 bp which represents $1/10^5$ of the total genome. For efficient amplification of a 1.5-kb *Bgl*II-generated fragment, it was necessary to prolong the extension time (72°C) from 1 up to 6 minutes. The longer extension time also allowed amplification of a 2.0-kb *Pvu*II and a 2.8-kb *Bam*HI-generated fragment, though at lower yield. We found that the addition of 1% formamide to the reaction mix improved the specificity of IPCR and less background amplification was observed. Instead of cloning, the IPCR product was purified by short reamplification (10 cycles) and then used for direct sequencing, Southern and Northern analysis, and screening of wild-type genomic and cDNA libraries.

References

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Identification and subcellular localization of neurophysin I and neurophysin II in the bovine adrenal medulla.

During the past years it has become apparent that oxytocin and vasopressin, originally described as the hormones of the posterior pituitary gland, are not confined to the hypothalamic-neurohypophyseal system. WEINDL & SOFRONIEW (1985) demonstrated their more widespread distribution throughout the central nervous system by immunocytochemical techniques as well as by radioimmunoassay. Neurohypophyseal hormones and the neurophysins, their related peptides, were also demonstrated biochemically and immunologically in the bovine adrenal medulla (ANG & JENKINS, 1984, HAWTHORN *et al.*, 1987) and their presence was postulated to have a regulatory function.

In this study the presence and subcellular localization of neurophysins in the adrenal medullary chromaffin cell is examined for the first time. Neurophysin I and II (bN_P I and bN_P II) were used as markers for respectively oxytocin and vasopressin. Upon differential centrifugation neurophysins can be enriched (1.3 times as compared to the total homogenate) in the crude organelle fraction.

After centrifugation of the crude organelle fraction through a linear sucrose density gradient 35 % of the bN_P I-immunoreactivity was present in the same fractions as the catecholamines. The other 65% sedimented in less dense fractions, indicating that part of the bN_P I-immunoreactivity is associated with another subcellular structure. The bN_P II-immunoreactivity, on the other hand, is almost completely restricted to the catecholamine-containing fractions.

The crude organelle fraction was also separated by successive centrifugation in self-generating Percoll-sucrose gradients into two subpopulations, one containing less dense adrenergic vesicles and the other the denser noradrenergic vesicles. From these experiments it can be concluded that both bN_P I and bN_P II are present in the adrenaline-containing fraction. Concerning the presence of neurophysins in the noradrenaline-containing fractions, however, no conclusion can be drawn since their concentrations are below the detection limit. Therefore the large granule fraction was also analysed by rate zonal centrifugation in hypertonic sucrose media (molarities ranging from 1.6 to 2.2 M). In this case noradrenaline-vesicles are preferentially enriched at high sucrose concentrations. From this experiment is can be concluded that bN_P I as well as bN_P II are also present in the noradrenaline-containing fractions although in a lower ratio as compared to the catecholamines.

We can conclude that : (i) neurophysins are present in the bovine adrenal medulla, (ii) bN_P II and part of bN_P I are predominantly in a soluble form in both the noradrenaline- and adrenaline-containing granules of the bovine chromaffin cells, (iii) another part of bN_P I is present in a subcellular structure, the nature of which remains to be investigated.

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