SUMMARY

The cooperation of pairs of proteins or the formation of large functional complexes of proteins is required for most if not all biological processes. Therefore, investigation of protein-protein interactions (PPI) within a cell is essential for the elucidation of biological processes and cellular networks. The two-hybrid system is the most commonly used method for PPI analyses. Hitherto, high-throughput analyses are almost exclusively performed in yeast, even when studying mammalian proteins. Putative interactions are subsequently confirmed in mammalian two-hybrid assays on a gene-by-gene basis.

The present work aimed at establishing a high-throughput, cost-effective method for analysing protein-protein-interactions directly in mammalian cells. This was achieved by combining mammalian two-hybrid system with transfected cell microarray to create the cell array-based protein-protein-interaction assay (CAPPIA). As for PCR- or oligonucleotide microarrays, the DNA samples were spotted and immobilized on glass slides in array formats. Each DNA spot contained bait and prey expression plasmids in addition to a reporter plasmid, which codes for an autofluorescent protein. After spotting the vector constructs, adherent human cells were added, creating a monolayer on the surface of the slides. Only cells growing on top of the DNA spots became transfected. In case of chimeric bait and prey protein interaction, the reporter gene was expressed, resulting in fluorescent reporter protein. Signals resulting from PPI were analyzed directly by fluorescence detection, without the need for further manipulation of the slides such as immunofluorescent staining or enzyme-based detection.

At first, production of the cell array slides and transfection conditions were optimised. Subsequently CAPPIA was shown to specifically and quantitatively detect protein-protein interactions in various mammalian cell lines. Moreover, screening of a small prey library against the human androgen receptor demonstrated that CAPPIA is well suited for the detection of hormone-dependent protein-protein-interactions. This was underscored by showing the dose-response of these interactions to androgenic compounds as well as to anti-androgenic reagents. Finally, it was shown that the possible combinatorial screens could be increased by application of slides without bait.

For this purpose microarrays consisted of spots containing one plasmid of a prey-library together with the reporter plasmid were designed. These so-called prey-reporter slides (PR-slides) were then analysed with cell lines that carried a stably or transiently expressed bait.

The high sample capacity of the cell arrays and the low reagent consumption make CAPPIA currently the most economical high-throughput detection assay for protein-protein interactions in mammalian cells. For this reason CAPPIA can become an important tool to increase the knowledge of the human interactome and thus of functional genomics.