Androgens are the key sex steroids of the male reproductive system in mammals and their action in target cells is mediated by high affinity, intracellular, androgen receptors (AR) which act directly in altering cellular gene expression. Since they play a pivotal role in regulating various aspects of cell metabolism, growth and differentiation, natural and synthetic androgens find use in various clinical situations including AIDS as well as cancer. However, long term steroid therapy has a potential disadvantage associated with unwanted side effects and to overcome the situation, there is a need for screening of potent steroids having a short half-life. Nevertheless, the development of a rapid, function based system to screen such natural and synthetic steroids for their biological activity has not received a great deal of attention.

Traditionally, the nature of ligand specificity and the relationship of ligand structure to steroid receptor activity have been studied in mammalian cells lacking endogenous receptors. For this, the generalized mechanism of hormone action has been utilized, whereby cotransfecting the receptor along with the reporter gene containing repeats of steroid response elements at the 5' end of the promoter allows for the subsequent monitoring of the reporter activity. Such expression systems have been further extended to non mammalian hosts and amongst them, yeast (*Saccharomyces cerevisiae*) seems to be the most popular since steroid hormones are gratuitous inducers of gene expression in yeast having little or no effect on the expression of endogenous genes. Hence, to analyze the functional complexity of steroid receptor signalling, we sought to develop a rapid and efficient genetic screen by capitalizing on the ability of rat AR to mediate hormone dependent transcriptional enhancement when expressed in *Saccharomyces cerevisiae*. A DNA cassette carrying three tandem repeats of the androgen response element (ARE) upstream of the yeast *CYC-1* promoter fused to the *E.coli lac Z* coding sequence (reporter) was targeted to integrate at the *ura-3-52* locus of the protease deficient yeast strain BJ 5460, to obtain strain YCR1. Southern hybridization results and analysis of genetic markers, confirmed the integration event.

YCR1 was utilized for developing a rapid and sensitive plate assay which would be suitable for screening the potential agonistic activity in natural or synthetic steroidal compounds. In reporter assays, this strain upon transformation with rat AR was inducible

over ~260-fold by androgen agonists (DHT, testosterone and R1881). Results from specificity studies and gel retardation assay were consistent with the observation that androgens have a direct transcriptional effect on the androgen receptors. Surprisingly, none of the antiandrogens (cyproterone acetate and flutamide) tested in competition assays were able to antagonize the DHT dependent induction of β-galactosidase activity. In gel retardation assays, exposure of receptors to agonist DHT *in vivo* led to the formation of hormone dependent protein-DNA complexes. Activation of rat AR by steroidal (cyproterone acetate) and non-steroidal (flutamide) antiandrogens, either alone or in combination with DHT also result in a similar pattern of migration for AR-ARE complexes . Additionally, LEM1, the ABC transporter that selectively modulates the biological potency of steroids in yeast, although proved to be operative in YCR1, was not responsible for the observed phenomenon. This evidence for antagonistic resistance in yeast suggests that antiandrogens exert their antagonistic effect at a step after receptor binds to DNA; possibly by interacting with yeast specific non receptor factors which may include either activator(s) or repressor(s). Therefore, study of transcriptional mechanism and pharmacology using this yeast based system would be helpful in identifying such factors and thus may help in unravelling the mechanism of steroidal resistance.

In addition to screening of ligands, the yeast based genetic screen is ideal for receptor structure-function studies. Mutations in the coding sequence of the AR result in a spectrum of endocrine disorders like androgen insensitivity syndrome (AIS), Refinstein syndrome and Kennedy's disease besides being associated with prostrate cancer and male breast cancer. With the objective of developing a detection method for early diagnosis of such function based mutations of the AR, the general properties of rat AR hormone binding domain in yeast were first analyzed. For this, *in vitro* site directed mutagenesis has been carried out for recreating known point mutants of rat AR (R757C, R814Q, V849M and V713M) whose transactivational properties have been well characterized in mammalian cells. Our results clearly demonstrated that receptor mediated signal transduction pathways for the mammalian AR has been conserved in *S. cerevisiae* even in the absence of an endogenous receptor. Having validated the yeast based genetic screen with known functional mutants, a library of random point mutations in rat AR HBD was generated by error-prone PCR. Instead of *in vitro* cloning into yeast expression vector we sought to utilize the power of yeast genetics for

the efficient *in vivo* cloning of the mutagenized PCR products into a specific gap-vector by homologous recombination. Using X-gal indicator plates containing DHT / other steroids, a subset of mutant receptors with distinct defects in steroid specificity and response was isolated and characterized. Of the thirty functionally defective mutants of rat AR characterized, eight representative mutants were further sequenced to pinpoint causative mutation(s). The canonical hormone binding domain structure of steroid hormone receptor superfamily was referred for determining the effect of these engineered mutations on protein structure. Results indicate that our system specifically detects mutations that disrupt function, avoiding analysis of functionally silent mutations not associated with receptor defect. Since this heterologous system is faithful in recapitulating the mammalian system, a similar strategy could be utilized for screening of mutations from patients samples by designing primers suitable for PCR/RT-PCR. Subsequently, *in vivo* gap repair by homologous recombination in yeast would allow direct functional assessment of endogenous rather than recreated mutant ARs. Additionally, utilizing a similar approach, the system would facilitate the complete mapping of mutations in the AR gene that impair normal receptor function.

It has become apparent in recent years that the activity of different transcription factors including steroid hormone receptors, can be regulated by their phosphorylation status. Our results from transactivation studies in yeast using modulators of protein phosphorylation, provide further evidence for the coupling of steroid response pathway with other cellular signal transduction mechanisms. Androgen regulated transactivation in yeast was found to be activated in a synergistic manner by activators of protein kinase-A pathway. The alternate signalling mechanism suggests a possible addendum to the classical mechanism of steroid hormone action which may explain the rapid effect of steroids. Since breast, endometrium, ovarian and prostrate cancer are characteristically associated with hormonal factors, such a cross-talk may have important implications on the pathophysiology of receptor associated diseases. Recapitulating this phenomenon in yeast provides us with an efficient means for the genetic analysis of different aspects towards a better understanding of the mechanism(s) of steroid hormone action. Thus, our heterologous expression system provides a general approach for dissecting the mechanism(s) of signalling by mammalian AR and may further facilitate the identification of various factors that participate in the process.