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ESTROGEN/ESTROGEN ANTAGONIST
REGULATION OF THE CELL CYCLE IN
BREAST CANCER CELLS

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INTRODUCTION

The role of estrogen in the growth of breast cancer was recognised over a century ago when it was shown that ovariectomy in premenopausal women with breast cancer resulted in tumor regression (Beatson 1896). Subsequent research showed that estrogen exerted its proliferative effects through a specific receptor (estrogen receptor-ER) and was essential for the initiation and progression of mammary cancer in experimental animals. This and other observations, such as the correlation between ER status of the tumor and a positive response to endocrine therapy, led to the development of estrogen antagonists (antiestrogens) for the treatment of breast cancer (Lerner and Jordan 1990). Tamoxifen, the antiestrogen most commonly employed in the treatment of hormone sensitive breast cancer, significantly decreases the rates of both disease recurrence and death (Early Breast Cancer Trialists' Collaborative Group 1992; Early Breast Cancer Trialists' Collaborative Group 1998; Fisher et al. 2001). However, tamoxifen therapy is limited by the frequent development of cellular resistance. In addition, synthetic non-steroidal antiestrogens like tamoxifen possess both estrogen agonist and antagonist activity and as such have the potential to induce proliferative side effects in other reproductive organs such as the endometrium (MacGregor and Jordan 1998). Due to these clinical limitations, more potent antiestrogens have been developed which do not have estrogen agonist properties, have prolonged effectiveness and are potentially efficacious in cancers that have developed resistance to nonsteroidal antiestrogens such as tamoxifen (Wakeling and Bowler 1987; Howell et al. 1995). This structurally distinct class of antiestrogens includes ICI 182780 (Faslodex) which is currently in clinical trials both as a primary treatment and for the treatment of tamoxifen resistant cancers (DeFriend et al. 1994; Howell et al. 1995). The mechanistic basis for the anti-tumour effects of antiestrogens is inhibition of estrogen
mediated mitogenesis, but the molecular events in antiestrogen induced growth arrest are not fully understood. Similarly, there is an incomplete understanding of the molecular events that mediate estrogen-induced mitogenesis in breast cancer cells. This chapter summarizes recent data from this and other laboratories on antiestrogen action in breast cancer cells and provides insight into the role of estrogen in mitogenic stimulation of target cells.

**EFFECTS OF ESTROGENS AND ANTIESTROGENS ON CELL CYCLE PROGRESSION**

Early information on the growth-inhibitory actions of antiestrogens originated from *in vitro* studies on breast cancer cell lines. These experiments suggested that growth rates (measured as changes in both cell number and tritiated thymidine incorporation into DNA) were significantly reduced by antiestrogen treatment (Lippman and Bolan 1975; Lippman et al. 1976). In the MCF-7 breast cancer cell model which has been the most widely studied experimental paradigm (Levenson and Jordan 1997), the typical growth inhibitory response to antiestrogens (both non-steroidal and steroidal antiestrogens) is a decrease in the proportion of cells synthesising DNA (S phase) after approximately 8 hours of antiestrogen treatment. This decrease in S phase coincides with an increase in the proportion of cells in Go/G1. It is clear that only cycling cells in early to mid G1 are sensitive to antiestrogens (Sutherland et al. 1983; Taylor et al. 1983; Reddel et al. 1984; Musgrove et al. 1989; Wakeling et al. 1989) which coincides with the period of the cell cycle when cells are sensitive to mitogenic stimulation.

The growth arrest following antiestrogen treatment of breast cancer cells has been used to study the effects of estrogen on cell cycle progression, since subsequent estrogen ‘rescue’ from antiestrogen-mediated growth arrest results in semi-synchronous progression of MCF-7 cells from Go/G1 through to S phase. The profile of such changes in cell cycle parameters is shown in Figure 1. This model has provided a robust experimental system to develop greater insight into the molecular events involved in mitogenic stimulation by estrogen in breast cancers.

**CELL CYCLE PROGRESSION**

Go/G1 to S phase progression is mediated by the action of a family of serine/threonine kinases, the cyclin-dependent kinases (CDKs), which in conjunction with their regulatory partners, cyclins, phosphorylate pRb and other members of the pocket protein family, p107 and p130 (Dyson 1998). The phosphorylation of pRb during G1 phase is mediated via two temporally distinct stages, in which initial phosphorylation by cyclin D1-Cdk4/6 is