Monoclonal rat antibodies to MCF-7 human breast cancer estrogen receptor (estrophilin) have been used to study the structure and cellular location of receptor in reproductive tissues and cancers as well as to develop assays for receptor that do not depend on the binding of hormone to the receptor protein. Fusion of splenic lymphocytes from Lewis rats, immunized with affinity-purified estrogen receptor from MCF-7 cell cytosol, with two different mouse myeloma lines [1-3], provided 13 cloned hybridoma cell lines; each of these (with one possible exception) secretes a unique idiotype of antibody that recognizes a distinct region of the receptor molecule. These antibodies have high affinity ($K_d=10^{-9}-10^{-10} \text{M}$) for both steroid-occupied and unoccupied estrogen receptor and recognize nuclear as well as cytosol forms of the receptor molecule. Although they vary in their cross reactivity with estrogen receptors from various animal species, each antibody appears to be completely specific for the 65,000-dalton steroid-binding subunit of the estrogen receptor complex, as judged by extensive sucrose gradient and immunoblot analyses of cytosol and nuclear extracts from a variety of tissues and cell lines. Cross reactivity patterns indicate both sequence homology and heterogeneity among mammalian and nonmammalian estrophilins. Some determinants (e.g., H222 and H226) are common to all tested estrogen receptors, including those from hen oviduct, whereas others (e.g., D547 and D58) are present only in mammalian receptors and one (D75) appears to be restricted to primate estrophilin.

As part of our ongoing effort to elucidate the distribution and dynamics of the estrogen receptor protein (ER) in target tissues, as well as to establish new criteria for the assessment of prognosis and hormonal response in breast cancer, we have developed an immunocytochemical assay (ER-ICA) for visualizing receptor directly in tissues and cells. Five monoclonal antibodies (D547, D58, D75, H222, H226) have been used individually to localize estrophilin by an indirect immunoperoxidase technique in frozen, fixed sections.
of human breast tumors, human uterus, rabbit uterus, and in other mammalian reproductive tissues, as well as in fixed MCF-7 cell cultures [4-6]. Specific immunoperoxidase staining for receptor in estrogen-sensitive tissues is confined to the nucleus of all stained cells, regardless of hormone status. Staining is absent in nontarget tissues, such as colon epithelium, and in receptor-negative breast cancers; in addition, it can be abolished by the addition of highly purified receptor to primary antibody. Heterogeneous staining has been observed in MCF-7 cells as well as in receptor-poor and receptor-rich breast cancers, possibly reflecting either variations in cell cycle or the presence of estrogen-sensitive and insensitive cells. Little or no cytoplasmic staining for estrophilin has been observed in any of the tissues or tumor cells examined thus far, including those deprived of exogenous estrogens. In a study of 117 human breast cancers by the ER-ICA method, the presence or absence of nuclear staining was significantly associated with the concentration of cytosolic estrogen receptor determined by steroid-binding assay. Staining intensity, epithelial cellularity and the proportion of tumor cells stained also correlated significantly with receptor concentration.

The heterogeneous pattern of nuclear staining observed in many of the breast tumors analyzed by the ER-ICA method may reflect the polyclonal origins of such tumors, and it is possible that the failure of some ER-rich tumors to respond to endocrine therapy results from the survival of a subpopulation of ER-poor cells. Although it is not possible to rule out experimental artifact as a factor in staining patterns, the correlation between the degree of heterogeneity and the levels of receptor determined biochemically suggest that the staining patterns reflect the true distribution of receptor-containing cells. Clinical response data will help resolve this issue. In a recent study of approximately 100 patients with stage 2 breast cancer, the most significant prognostic indicator was the percentage of tumor cells that displayed nuclear staining for estrophilin by the ER-ICA method.

In view of the exclusively nuclear localization of specific immunoperoxidase staining for receptor in all estrogen-sensitive tissues and cells studied thus far, it appears that both cytosol and nuclear forms of the receptor may reside in the nuclear compartment in the presence and absence of steroid. We have seen little or no increase in nuclear staining intensity in MCF-7 cells and in uteri from immature rabbits or from postmenopausal women following short-term exposure of cells or tissue to estradiol. These