Subcellular distribution of oestrogen receptors

TWO studies have been reported repudiating the long-held dogma that, in the absence of oestrogen, the oestrogen receptor is confined to the cytosol[1,2]. The new model[1-3] supplants this interpretation with one in which unfilled receptor occurs exclusively in the nuclear compartment. Unfortunately, the new model may be just as full of artefacts as the old (see refs 4, 5 and below).

First, the new model, admittedly '... based on limited data and ... therefore rather speculative ...'[3], fails to take account of approximately 100 publications demonstrating, by methods including analytical subcellular fractionation, affinity binding to intact cells and photoaffinity procedures, intrinsic localization of receptors for steroid hormones in extranuclear membranes of target cells, including the plasmalemma (from which the macromolecules are extracted by the conventional methods of preparing cytosol). This body of evidence has been reviewed extensively[6-9]. The Gorski group dispenses with these (uncited) potentially contradictory observations by the unsupported assertion that neither their laboratory[2] nor that of King and Greene[1] see "... evidence of binding [*sic*] of receptors to plasma membranes, a site that has been suggested in the past"[3].

Second, the immunocytochemical distribution of 70–95% of oestrogen receptor with monoclonal antibody in (or on?)[4] the nucleus, without cytoplasmic staining[1], in uteri of immature or pseudopregnant rabbits or of rabbits only 8 days after ovariectomy, and in a mammary tumour from a postmenopausal woman, is in direct contradiction[4] to reports from the same laboratory within the past 18 months[10]. Since the fixation methods were essentially equivalent (L. Zamboni, personal communication), this variable seems excluded as the basis for the complete turnabout. The remaining differences between the successive observations were the use in the current work[1] of frozen sections, notorious for introduction of membrane distortion, and a different monoclonal antibody. It is difficult to assess the relative contribution of these additional variables. Alternative fixation-processing methods may well be required for good preservation of cell ultrastructure in the extranuclear regions without sacrifice of immunoreactivity.

Third, in the case of the report from the Gorski laboratory, there are abundant indications that the nuclear localization of 'unfilled' receptor was associated with profound perturbations of the native state of the GH_3 tumour cells used as oestrogen targets. Thus, the time-consuming enucleation process[11] on which the conclusions rest was carried out on Percoll-density-selected cells that were then preincubated at 37 °C for 45 min in the presence of purportedly subtoxic concentrations of cytochalasin B and dimethyl sulphoxide, followed by 45 min centrifugation at 37 °C in a shearing gradient of Percoll and additives[11]. The antibiotic[12], its solvent[13], the Percoll itself[14,15], as well as the elevated temperature[7], required to separate the major cellular compartments, are each known to perturb the cell surface. The increased endocytotic activity associated with such nonspecific stimuli, especially in tumour cells exhibiting an unusually high degree of basal pinocytosis, is well documented[7]. Nuclear translocation of resultant vesicles or macromolecular complexes by specified routes[7] would be expected to promote concentration of receptor from plasmalemmal and other extranuclear sources.

Fourth, in the above work, the relative integrity of the nucleoplast fraction[2] was, surprisingly, not established by analyses for enzyme markers characteristic of other cellular compartments. Indeed, it is generally recognized that extranuclear membranous material is closely associated with the outer nuclear envelope on separation of the latter organelle by several procedures, even surviving shearing through heavy sucrose. Thus, removal of the outer nuclear membrane is required for definitive studies of nuclear composition (ref. 16 and citations therein). By inspection of the stained preparation[2], as well as by definition[3], the nucleoplast, consisting of '... nuclei plus a small amount of cytoplasm plus intact membrane ...,' is an incompletely characterized fraction.

Fifth, although, at first glance, the analogy drawn by the recent papers[1-3] to 'unfilled' receptors for thyroid hormone being concentrated in the nuclear fraction of its target cells seems compelling, neither group takes note of the equally conclusive data on the initial binding of triiodothyronine to the cell surface, followed by clustering and internalization, apparently in vesicular form[9,17]. Similar observations are available for specific surface binding of other relatively hydrophobic agonists, including ouabain[18] and oestradiol-17β (refs 6, 19). These findings bear a close analogy to the emerging data for peptidal effectors[7]. Indeed, if primary recognition of blood-borne oestrogen were to be effected only within the confines of the nucleus, one is left to ponder the nature of the long-range forces that could underlie such a remarkable sensing and migration mechanism in 'target' cells.

Finally, we welcome as long overdue[6] critical re-evaluation of the unmodified, traditional model of cytosolic localization of unbound receptor for steroid hormones, useful as it has been. However, it would seem prudent not to throw out the baby with the bathwater.

So McClellan *et al.*[20] have failed to present data on localization of oestrogen receptors in ovariectomized monkeys. In a personal communication that we have her permission to cite, McClellan has stated that 'there was cytoplasmic staining in spayed controls, but it was not well differentiated from background', and that, presumably for the above reason, their group had 'postponed work on ovariectomized monkeys for more detailed study'. As is widely acknowledged, it is necessary to use great vigilance to combat the growing problem of contamination of animal quarters with even those low levels of oestrogen that promote nuclear association of transformed receptor.

Clearly, much remains to be done before the problem of subcellular distribution of oestrogen receptor in normal target cells in the absence of hormone can be firmly resolved.

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GREENE AND KING REPLY—Although an initial report[1] from this laboratory contained micrographs showing immunocytochemical localization of oestrogen receptor in the cytoplasm of tumour cells from paraffin-embedded breast cancers, we later found that this staining was not specific and could be mimicked by several unrelated polyclonal and monoclonal rat antibodies. In addition, essentially all cytoplasmic staining could be abolished by including suitable carrier proteins (for example, 10% normal goat serum) in the antibody solutions. Extensive work by us and others[2-6] since then, with 10 unique monoclonal antibodies to human oestrogen receptor, indicates that specific staining for the receptor is confined to the nuclei of target cells in all conditions studied. These include at least 20 different