

Characterization of two Ah receptor forms in HeLa cells.

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This study was initiated to identify whether more than one form of the Ah receptor (AhR) exists and their possible functional significance in AhR activity. Monoclonal antibodies have been developed against an N-terminal peptide which are highly specific against the Ah receptor (AhR) on western blots and allow detection of the AhR in the absence of ligand. These antibodies are able to recognize the mouse, rat or human AhR. Cytosolic preparations from HeLa cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer of the protein to PVDF membrane. The AhR was visualized using monoclonal antibody Rpt 1 and goat-anti-mouse peroxidase conjugate. Two bands were detected at 104 and 106 kDa in approximately a one to one ratio. The photoaffinity ligand, 2-azido-3-[<sup>125</sup>I]iodo-7,8-dibromodibenzo-*p*-dioxin, was added to HeLa cells in culture, after 1 h the cells were rinsed with phosphate buffered saline and UV irradiated<sup>1</sup>. A cytosolic extract and a high salt nuclear extract were isolated and subjected to SDS-PAGE followed by transfer of the protein to PVDF membrane. The AhR was visualized on the membrane revealing two bands in the cytosolic fraction. The membrane was subjected to autoradiography, after alignment of the film with the membrane only the 106 kDa (upper) band was photoaffinity-labeled. Examination of the nuclear fraction revealed only the photoaffinity-labeled 106 kDa form of the AhR. This would indicate that in HeLa cells the AhR exists in two forms a ligand binding form and a nonligand binding form. The 104 kDa AhR does not appear to be a proteolysis product of the 106 kDa due to the absence of photoaffinity ligand in the 104 kDa band. In addition, upon exposure to a AhR ligand only the 106 kDa form translocates into the nucleus. Sucrose density gradient analysis of HeLa cell cytosol indicated that both forms cosedimented at ~ 9S. Both forms are also immunoprecipitated with an anti-90 kDa heat shock protein antibody (8D3). These results would indicate that the two forms of the AhR exist in HeLa cells in a 9S complex containing the 90 kDa heat shock protein. The presence of two forms of the AhR has also been detected in other human cell lines (e.g. A431 squamous cell carcinoma line). The structural difference between the two forms of the AhR is currently under investigation.

<sup>1</sup>Perdew, G.H. Comparison of the Nuclear and cytosolic forms of the *Ah* receptor from Hepa 1c1c7 cells: charge heterogeneity and ATP binding properties. *Arch. Biochem. Biophys.* 1991;291:284-9.

