0026-895X/07/7101-386–387$20.00
MOLECULAR PHARMACOLOGY
Vol. 71, No. 1
31971/3165413

Mol Pharmacol 71:386–387, 2007
Printed in U.S.A.

Response to Comments on "Calpain Mediates the Dioxin-Induced Activation and Down-Regulation of the Aryl Hydrocarbon Receptor"

Received October 19, 2006; accepted October 19, 2006

In response to the comments by Dr. Richard S. Pollenz in reference to our article (Dale and Eltom, 2006), we provide the following points for discussion.

Indeed, our study has built on some significant reports published by leading scientists in the AhR field, as outlined in our manuscript.

Calpain-catalyzed proteolysis is typically limited, resulting in a clipped but substantially intact protein, and Suzuki et al. (1988) have proposed that limited proteolysis by calpain could render substrate proteins destabilized and susceptible to a variety of other cellular proteases, including proteasomes. In this regard, many regulatory cellular proteins have been reported to be regulated by both calpain and proteasomes. For example, cyclin D1 was shown to be regulated by calpain (Choi et al., 1997) and proteasome (Diehl et al., 1998; Guo et al., 2005). However, none of these reports has attempted to reconcile the apparent discrepancy.

In the case of AhR, we do have evidence for ~90- and 70-kDa calpain-generated fragments from the mouse AhR^{b-1}, which has a full length of 95 kDa. These data and further investigational studies were beyond the scope of the published report, but they are currently in preparation for publication.

On the other hand, despite multiple reports from Dr. Pollenz's laboratory over the last decade on the ubiquitin-proteasome-dependent degradation of AhR, not a single piece of evidence for ubiquitinylated AhR forms has been presented in these studies (Pollenz, 1996; Davarinos and Pollenz, 1999; Song and Pollenz, 2002, 2003; Wentworth et al., 2004)

According to our model, we proposed that after the rise in intracellular calcium (by the AhR ligands or other agents), calpain is activated to partially cleave the AhR at its far-end C-terminal putative site (resulting in the ~90 kDa). This truncated form, which is transcriptionally active, readily translocates to the nucleus to heterodimerize with ARNT and to bind DNA. After its transcriptional activation of responsive genes, the receptor is shuttled to the cytoplasm, where further cleavage by calpain results in 70-, 55-, and 37-kDa fragments. It is plausible that these smaller fragments are substrates for ubiquitinylation and further degradation by proteasome. Interestingly, in Ma and Baldwin (2000), the ubiquitinylated proteins immunoprecipitated by anti-AhR contain, in addition to the high molecular mass complexes of the full length AhR, some heavily stained bands migrating at 70 kDa in both TCDD-treated and untreated lanes.

It is noteworthy that, in a recent work from Pollenz's laboratory (Pollenz et al., 2005), he reports that two truncated forms of AhR (AhR_{500} and AhR_{640}) lacking the C-terminal domains, which were expressed exogenously, have exhibited faster degradation rates than endogenous full-length AhR and are insensitive to both RNA and protein synthesis inhibitors. He concluded that "the degradation mechanism is more complex than originally thought and appears to involve two distinct pathways that function under different situations." It is conceivable that by lacking the C-terminal domain, these forms do not require calpain cleavage and are directly degraded by proteasomes

Further support for our contention came from a recent work from Gary Perdew's laboratory (J. L. Morales and G. H. Perdew, unpublished observations). These researchers proposed that proteasomes might not be the only pathway of degradation and that the calpain family might participate at least in CHIP-mediated human AhR degradation.

As far as the correlation between C-terminal truncation of AhR and nuclear translocation and/or gene activation, our proposal is in agreement with a published report from Perdew's laboratory (Kumar et al., 2001), in which they showed that C-terminal truncation of AhR in human cell lines leads to an increase in gene activation greater than that seen with the full-length AhR. In fact, Pollenz et al. (2006) illustrated that "truncation of the C-terminal 305 amino acids of the Ah^{b-1} receptor (AhR_{500}) results in protein that exhibits predominantly nuclear localization."

In closing, our manuscript reported crucial findings, which we attempted to interpret within the context of relevant data in the literature while leaving some room for further interpretation by other investigators who are making parallel contributions. It is our anticipation that our report will shed some light on critical phenomena reported in the AhR field without satisfactory explanation.

Division of Cancer Biology,
Department of Biomedical Sciences,
Meharry Medical College,
Nashville, Tennessee
E-mail: seltom@mmc.edu

SAKINA E. ELTOM
YOLANDA DALE

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.106.031971.

References

Dale YR and Eltom SE (2006) Calpain mediates the dioxin-induced activation and down-regulation of the aryl hydrocarbon receptor. *Mol Pharmacol* **70:**1481–1487.
Choi YH, Lee SJ, Nguyen P, Jang JS, Lee J, Wu ML, Takano E, Maki M, Henkart

ABBREVIATIONS: Ahr, aryl hydrocarbon receptor.

PA, and Trepel JB (1997) Regulation of cyclin D1 by calpain protease. *J Biol Chem* **272:**28479–28484.

Davarinos NA and Pollenz RS (1999) Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. *J Biol Chem* **274:**28708–28715.

Diehl JA, Cheng M, Roussel MF, and Sherr CJ (1998) Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* **12:**3499–3511.

Guo Y, Yang K, Harwalkar J, Nye JM, Mason DR, Garrett MD, Hitomi M, and Stacey DW (2005) Phosphorylation of cyclin D1 at Thr 286 during S phase leads to its proteasomal degradation and allows efficient DNA synthesis. *Oncogene* **24:**2599–2612.

Kumar MB, Ramadoss P, Reen RK, Vanden Heuvel JP, Perdew GH (2001) The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity. *J Biol Chem* **276:**42302–42310.

Ma Q and Baldwin KT (2000) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced degradation of aryl hydrocarbon receptor (AhR) by the ubiquitin-proteasome pathway. Role of the transcription activation and DNA binding of AhR. *J Biol Chem* **275:**8432–8438.

Pollenz RS, Popet J, and Dougherty EJ (2005) Role of the carboxy-terminal transactivation domain and active transcription in the ligand-induced degradation of the mouse Ah^{b-1} receptor. *Biochem Pharmacol* **70:**1623–1633.

Pollenz RS, Wilson SE, and Dougherty EJ (2006) Role of endogenous XAP2 protein on the localization and nucleocytoplasmic shuttling of the endogenous mouse Ah^{b-1} receptor in the presence and absence of ligand. *Mol Pharmacol* **70:**1369–1379.

Song Z and Pollenz RS (2002) Ligand dependent and independent modulation of AH receptor localization, degradation, and gene regulation. *Mol Pharmacol* **62:**806–816.

Song Z and Pollenz RS (2003) Functional Analysis of murine aryl hydrocarbon (AH) receptors defective in nuclear import: impact of receptor degradation and gene activation. *Mol Pharmacol* **63:**597–606.

Suzuki K, Imajoh S, Emori Y, Kawasaki H, Minami Y, and Ohno S (1988) Regulation of activity of calcium activated neutral protease. *Adv Enzyme Regul* 1988;27:153–69.

Wentworth JN, Buzzeo R, and Pollenz RS (2004) Functional characterization of aryl hydrocarbon receptor (zfAHR2) localization and degradation in zebrafish (*Danio rerio*). *Biochem Pharmacol* **67:**1363–1372.