MOLECULAR BIOLOGY OF THE AH RECEPTOR AND AH RECEPTOR-DEPENDENT SIGNALING

MOLECULAR CLONING OF THE HAMSTER AH RECEPTOR REVEALS A UNIQUE Q-RICH SUBDOMAIN STRUCTURE

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Introduction

Dioxins and related halogenated aromatic hydrocarbons are ubiquitous environmental contaminants. Evaluation of the risks posed by these compounds to humans is hampered by the exceptionally large inter- and intraspecies differences occurring in laboratory animals to some of their effects. These differences culminate in acute lethality: to the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the hamster is about 1000-fold more resistant than the guinea pig and a difference of the same magnitude exists between a TCDD-sensitive rat strain, Long-Evans (*Turku AB*) and a TCDD-resistant strain, Han/Wistar *(Kuopio*) [H/W]. By contrast, both H/W rats and hamsters are susceptible to enzyme induction, thymus atrophy and fetotoxicity by TCDD[1].

Most of the biological effects of dioxins are mediated by the AH (aryl hydrocarbon) receptor (AHR). The AHR is a ligand-activated transcription factor belonging structurally to basic helix-loop-helix (bHLH)/PAS proteins, a family of molecules encompassing important regulators of biological rhythms and of CNS development as well as transcriptional coactivators[2]. The molecular mechanism of AHR action has so far been only elucidated for CYP1A1 induction, but this is believed to be a general mode of gene regulation by the AHR. In an inactive state, the AHR is located in the cytoplasm in a protein conglomerate along with two molecules of the chaperone hsp90, certain immunophilins and c-src. Binding of ligand such as TCDD results in translocation of the AHR into the nucleus and disruption of the complex. Inside the nucleus, the AHR dimerizes with a related bHLH/PAS protein, ARNT, and then binds to DNA at specific sites containing a consensus hexanucleotide core. These dioxin response elements act as enhancers for genes regulated by dioxins. Since the enhancer sites are usually situated relatively far upstream of the gene promoter, gene activation by dioxins probably involves nucleosomal disruption and interaction with transcriptional coactivators and/or corepressors[3].

The AHR protein consists of distinct functional modules. The bHLH domain located in the N terminus is responsible for DNA binding and heterodimerization. The PAS motif flanking the bHLH structure affords specificity to dimerization and also contains most of the ligand-binding domain. The C terminus comprises a potent transactivation domain composed of several

interacting subdomains, one of which is a glutamine-rich subunit. This has been shown to be essential for the transactivation function of the AHR *in vitro*[3,4].
Recent cloning of the H/W rat AHR cDNA in our laboratory disclosed two point mutations affecting the receptor primary structure. One of the mutations is located in a highly variable region and is probably of minor, if any, biological significance. The other mutation resides in the first invariant nucleotide of intron 10 leading to altered splicing of the mRNA. This gives rise to two different proteins, both of which are smaller than the wild-type rat receptor and bear unique transactivation domains[5]. Genetic studies implied that the altered AHR is the major determinant of TCDD resistance in H/W rats[6].

The findings in H/W rats prompted us to analyze the primary structure of the hamster AHR. Our working hypothesis was that the peculiarly selective responsiveness to TCDD of H/W rats and hamsters is largely due to the structure of their AHR transactivation domains.

Methods and Materials
We used RT-PCR cloning for the endeavour. Total RNA was isolated by the method described by Chomczynski[7] from the liver of a young adult female and a young adult male hamster. cDNA was generated with an oligo-dT primer and AMV reverse transcriptase. The first primers for PCR were selected from highly conserved regions towards the 5' end of the open reading frame. The fragment thus produced was cloned into the pCR-Script SK(+) AMP vector (Stratagene) by blunt-end cloning and its primary structure was resolved by automatic sequencing. The sequence information obtained was utilized in designing the next forward PCR primer and a suitable reverse primer site was sought from conserved stretches downstream. By this means, the body of the coding region was cloned in 5 fragments. To get the ends of the coding segment, modified 5' and 3' race PCRs were used. For the 5' end, total RNA was first reverse transcribed with a gene-specific primer-1. Single-stranded cDNA was then purified and poly-A –tailed with terminal deoxyducleotidyl transferase. The new 3' tail created was used for annealing a modified anchor primer in a PCR reaction run with a nested gene specific primer-2. The 5' end of the forward anchor primer was rich in GC and this stretch was exploited in a final PCR-reaction with a nested gene specific primer-3. To obtain the 3' end of the open reading frame, single-stranded cDNA was first generated with the same anchor primer described above. Three semi-nested PCR reactions were then run with an oligo complementary to the GC-rich 5'end of the anchor primer as the reverse primer and three nested oligos from the extreme 3' end of the resolved coding sequence as forward primers.

To decrease the frequency of Taq polymerase-induced errors during PCR reactions, we used a high–fidelity enzyme blend (DyNazyme EXT DNA polymerase; Finnzymes). All ambiguities were resolved by auxiliary clones, and the entire coding region was sequenced from two animals with 100% identical results.

Results and Discussion
The cDNA sequence for the coding region of the hamster AHR gene revealed that the translated protein would be somewhat larger than the wild-type rat receptor (calculated sizes 103.3 and 96.2 kDa for hamster and rat receptors, respectively). This is in keeping with previous immunoblot data, although SDS-PAGE yielded larger absolute sizes for the receptors[8]. The N terminal end of the hamster receptor was highly conserved, and the size difference turned out to be solely due to a

conspicuous change in the C terminus of the hamster AHR. Because of incorporation of short repeats of satellite-like DNA towards the 3'end of the coding region, the C terminus of the translated protein would have some 60 extra amino acids compared with the corresponding region

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HamsterAHR 475 PLDSHFLT-HGSEGDGWQDSIASIGSEAELKHEQIGHGQDMNPAVSGGPPGLFPDNRNSD 533
SDRatAHR   475 .......MDSM..CGS..G.F.VASN..L....E.R.T..V.LTL....SE.....K.N. 534
B6MouseAHR 471 L......MGSV.KCGS....F.AA....A........A..V.L.L....SE.....K.N. 530
HumanAHR   480 .FENN.FNESMN.CRN...NT.PM.NDTI......DQP..V.-SFA..H....Q.SK... 538

HamsterAHR 534 LYSIMKNLGIDFDDIKRMQSEEFFRTEL--AGEVDFRDIDITDEILTYVQDSLNRSTLLS 591
SDRatAHR   535 .....R......E..RS..N......DS--S.....K.................N....N 592
B6MouseAHR 531 .....R......E..RS..N......DSTA......K.................N....N 590
HumanAHR   539 ............E..RH..N.K...NDF--S..... ...L............SK.PFIP 596

                                                  Q-rich
HamsterAHR 592 SASQQQP-VTQHLSCMLQERLHLGQRQLQQHETQAAEPQQQLGHQTAPQQELCHQTAPQQ 650
SDRatAHR   593 ..C....-.S...........Q.E.--------------....Q-.QH.T---------- 626
B6MouseAHR 591 ..C....-............Q.E.--------------....Q-.PPP---------- 623
HumanAHR   597 .DY...QSLALNS...V..H...E.--------------...HHQKQVV---------- 631

                                                  Q-rich
HamsterAHR 651 QMCLQMAPQQELCHQMEPQQQLCLQMAPQQQLCHQTAPQQQLCLQMAPQQELCHQTAPQP 710
SDRatAHR   627 --------------QTL...R...QVEV..H------------------------------ 643
B6MouseAHR 624 --------------QAL.......QMVC...------------------------------ 640
HumanAHR   632 ---------------V.......QK.K-------------------------------- 643

                                                  Q-rich
HamsterAHR 711 ELGQKMNHAQVNGMFASWNPTPLVPFSCPQQELKHYDVFSDLQGAIEEFPYKSEMDSMPY 770
SDRatAHR   644 .....TK.M...........A.P.S.......R...SL..G...TAQ.......V..... 703
B6MouseAHR 641 D..--PK.T.I..T........P.S.N.........QL..S...TAQ.......V..V.. 698
HumanAHR   644 -------.M......EN..SNQF...N....DPQQ.N..T..H.ISQ.............. 696

HamsterAHR 772 TQSFAPCNQSVLPQRSKCAQLDLPGKGFEPSLHPNTSNVGDFVTCLQVPENQRHEVHPQS 830
SDRatAHR   704 ..N.......L..EH..GT...F..RD..R.....A..LE...S..........GINS.. 763
B6MouseAHR 699 ..N......PL..EH..SV...F..RD.......T...LD-FVS........S.GINS.. 757
HumanAHR   697 ..N.IS...P....H...TE..Y.MGS....PY.T..SLE.......L....K.GLN... 756

HamsterAHR 832 AMVAPQTYYAGAMSMYQCQPGPQHVPVEQMQYSPAVPDSQAFLNKFQNQGVLNETYSSEL 890
SDRatAHR   764 ...S..A............A....T..D..H...EI.G.....S...SPSI...A..AD. 823
B6MouseAHR 758 ...S..A..............RT..D.T...SEI.G.....S.V.S------------ 805
HumanAHR   757 .IIT...C....V.......E...TH.G....N.VL.GQ.........-........PA.. 815

HamsterAHR 892 NSVGHRQTTAHLH---HPAEGRPFPDITPSGFL 920
SDRatAHR   824 S.I..L..A...P---RL..AQ.L......... 853
B6MouseAHR 805 ------------------------------- 805
HumanAHR   816 .NINNT...T..QPLH..S.A.....L.S.... 848
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Fig 1. Comparison of hamster, rat, mouse and human AHR sequences in the C-terminal transactivation domain. The glutamine-rich region is denoted. Identical amino acids are shown with dots and missing amino acids with hyphens.

in the rat wild-type AHR (Fig. 1). The insertion localizes in the transactivation domain of the AHR. More specifically, the change affects the glutamine-rich subdomain of the receptor so that the number of glutamine residues in hamster AHR is almost twice as high as in its mouse, rat and human counterparts (Table 1). This subdomain has previously been shown to be a potent functional modulator of the AHR[4]. It has also recently been found to contain a binding site for retinoblastoma protein, an important regulator of cell cycle[9].

Table 1. Number of glutamine residues in the glutamine-rich subdomain

Animal	Glutamine
Hamster	49
Rat	28
Mouse	27
Human	25

It is not yet known to what extent the unique glutamine-rich subdomain structure accounts for the peculiarly selective responsiveness of hamster to TCDD. It is intriguing to note, however, that the two laboratory animals most resistant to the acute lethality of TCDD, hamsters and H/W rats, both harbor an AHR with a remodelled transactivation domain. Furthermore, animals extremely susceptible to TCDD toxicity, fish, have an AHR that lacks the glutamine-rich subdomain[10]. Therefore, it will be exciting to see how the receptors of hamsters and H/W rats compare with the "standard" AHRs in functional tests.

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