

Human, mouse and rat HNF4 $\alpha$ 2 amino acid sequence alignment

Human 1 56  
MRLSKTLVDMMDADYSAALDPAYTTLEFENVQVLTMGNDTSPSEGTINLNAPNSLGVLSALCAICGD  
.....

Mouse  
MRLSKTLAGMDMADYSAALDPAYTTLEFENVQVLTMGNDTSPSEGANLNSSNSLGVLSALCAICGD  
.....

Rat  
MRLSKTLADMDMADYSAALDPAYTTLEFENVQVLTMGNDTSPSEGANLNSSNSLGVLSALCAICGD  
.....

Human 121  
RATGKHYGASSCDGCKGFFRRSVRKNHMYSRFSRQCVVDKDKRNQCRYCRLKKCFRAGMKKEAV  
.....

Mouse  
RATGKHYGASSCDGCKGFFRRSVRKNHMYSRFSRQCVVDKDKRNQCRYCRLKKCFRAGMKKEAV  
.....

Rat  
RATGKHYGASSCDGCKGFFRRSVRKNHMYSRFSRQCVVDKDKRNQCRYCRLKKCFRAGMKKEAV  
.....

Human H1 H3 186  
QNERDRISTRSSYEDSSLP SINALLQAEVLSRQITSPVSGINGDIRAKKIASIADVCE SMKEQL  
.....

Mouse  
QNERDRISTRSSYEDSSLP SINALLQAEVLSQQITSPISGINGDIRAKKIANITDVCE SMKEQL  
.....

Rat  
QNERDRISTRSSYEDSSLP SINALLQAEVLSQQITSPISGINGDIRAKKIANITDVCE SMKEQL  
.....

Human H4-H5 R226 251  
LVLVEWAKYIPAFCELP LD DQVALLRAHAGEHLLLGATKRSMVFK DVLLLGNDYIVPRHCPEL LAE  
.....

Mouse  
LVLVEWAKYIPAFCELLDDQVALLRAHAGEHLLLGATKRSMVFKDVLLLGNDYIVPRHCPEL LAE  
.....

Rat  
LVLVEWAKYIPAFCELLDDQVALLRAHAGEHLLLGATKRSMVFKDVLLLGNDYIVPRHCPEL LAE  
.....

Human V255 H7 H8 H9 316  
MSRVSIRILDELVLPFQELQID DNEYAYLKAI IFFDPDAKGLSD PGKIKRLRSQVQSLEDYIND  
.....

Mouse  
MSRVSIRILDELVLPFQELQIDDNEYAYLKAI IFFDPDAKGLSDPGKIKRLRSQVQSLEDYIND  
.....

Rat  
MSRVSIRILDELVLPFQELQIDDNEYAYLKAI IFFDPDAKGLSDPGKIKRLRSQVQSLEDYIND  
.....

Human H10-H11 H12 381  
RQYDSRGRFGELLLLLPTLQSI TWQMIEQIQFIKLF GMAKIDNLLQEMLLGGSPSDAPHAHHPLH  
.....

Mouse  
RQYDSRGRFGELLLLLPTLQSI TWQMIEQIQFIKLF GMAKIDNLLQEMLLGGSPSDAPHAHHPLH  
.....

Rat  
RQYDSRGRFGELLLLLPTLQSI TWQMIEQIQFIKLF GMAKIDNLLQEMLLGGSPSDAPHAHHPLH  
.....

Human  $\alpha$ 2 insert 446  
PHLMQEHMGTNVIVANTMP THLSNGQMCEWPRPRGQAATPETPQSPSPGSGSEPYKLLPGA VAVAT  
.....

Mouse  
PHLMQEHMGTNVIVANTMP SHLSNGQMCEWPRPRGQAATPETPQSPSPGSGSESYKLLPGA IITTT  
.....

Rat  
PHLMQEHMGTNVIVANTMP SHLSNGQMCEWPRPRGQAATPETPQSPSPGSGSESYKLLPGA IITTT  
.....

Human 465  
IVKPLSAIPQPTITKQEVI  
.....

Mouse  
IVKPPSAIPQPTITKQEAI  
.....

Rat  
IVKPPSAIPQPTITKQEAI  
.....

**Figure S5. Alignment of human, mouse and rat HNF4 $\alpha$ 2 amino acid sequence.**

In this study, we determined the ligand bound to three different species of HNF4 $\alpha$  – human (Fig. S3), mouse (Fig. 3) and rat (Fig.s 1,2). The ligand binding domain (LBD) of rat and mouse HNF4 $\alpha$  is 100% identical on the amino acid level. The LBD of rat/mouse HNF4 $\alpha$  is 97.5% identical to human; there are only 6 amino acids that are different, none of which contact the ligand [1,2]. The DNA binding domain (DBD) of human, mouse and rat HNF4 $\alpha$  are 100% identical. Indicated is the conventional amino acid numbering for HNF4 $\alpha$ 2, which does not include an N-terminal extension resulting from an alternative translation start site (underline) [3]. HNF4 $\alpha$ 1 (used in Fig.s 5,6) is identical to HNF4 $\alpha$ 2 except for the  $\alpha$ 2 insert (double underline). Highlight: black, species differences; red, ligand contacts; yellow, DBD; green, LBD helices; blue, LBD  $\beta$ -sheets. Residues mutated in this study (V255, R226) are noted. Human HNF4 $\alpha$ 2 (NM\_000457.3); mouse HNF4 $\alpha$ 2 (NM\_008261); rat HNF4 $\alpha$ 2 (NM\_022180).

1. Dhe-Paganon S, Duda K, Iwamoto M, Chi YI, Shoelson SE (2002) Crystal structure of the HNF4 alpha ligand binding domain in complex with endogenous fatty acid ligand. *J Biol Chem* 277: 37973-37976.
2. Wisely GB, Miller AB, Davis RG, Thornquest AD, Jr., Johnson R, et al. (2002) Hepatocyte nuclear factor 4 is a transcription factor that constitutively binds fatty acids. *Structure* 10: 1225-1234.
3. Sladek FM, Zhong WM, Lai E, Darnell JE, Jr. (1990) Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev* 4: 2353-2365.