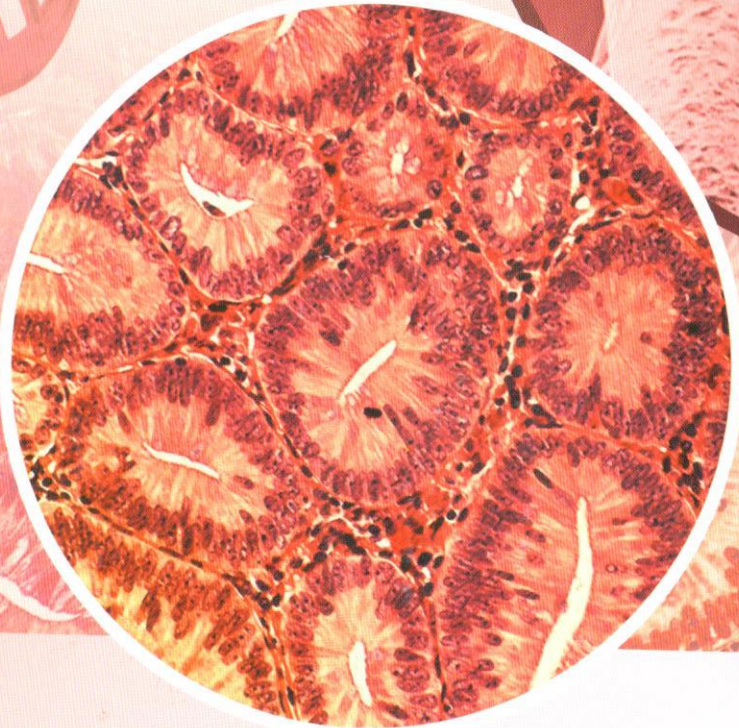


2013. május 29 - 30.

STRAUB-NAPOK

MEGHÍVÓ



MTA Szegedi Biológiai Kutatóközpont

Az Európai Unió Kiválósági Központja

Szeged, Temesvári krt. 62., Nagyelődő



Molecular and functional heterogeneity of honeybee hemocytes

Erika Gábor^{1,2}, Tibor Török², Gábor Csordás¹, Viktor Honti¹,
Éva Kurucz¹ and István Andó¹

(¹Institute of Genetics, BRC HAS, Szeged;

²Department of Genetics, University of Szeged, Szeged)

In situ dissection of embryonic enhancers flanking the bxd maintenance element of *Drosophila*

Viktória Kiss and László Sipos

(Institute of Genetics, BRC HAS, Szeged)

Identification of an adult segment-specific enhancer in *Drosophila melanogaster*

Péter Kaltenecker and László Sipos

(Institute of Genetics, BRC HAS, Szeged)

In vivo analysis of Spectraplakin function in *Drosophila* wound closure

Dániel Danszky, Ferenc Jankovics and Miklós Erdélyi

(Institute of Genetics, BRC HAS, Szeged)

Orthologs of the rabbit *Oct4*, *Sox2* and *Klf4* pluripotency genes

Flóra Vajda, Viktória Szabó, Diána Nagy, Anita Káity-Szécsényi and
Melinda Pírity

(Institute of Genetics, BRC HAS, Szeged)

Lack of PRC1 member *Rybp* leads to accelerated stem cell proliferation

Tünde Berecz, Bea Militár and Melinda Pírity

(Institute of Genetics, BRC HAS, Szeged)

***Rybp* null ES cells exhibit reduced neurogenic potential**

Gergő Kovács¹, Olga Ujhelly², Bea Militár¹, Nuttha Klincumhom²,
Sára Berzsényi², András Dinnyés² and Melinda Pírity¹

(¹Institute of Genetics, BRC HAS, Szeged;

²Bio Talentum Ltd., Gödöllő)

Identification and characterization of genes involved in embryonic dorsal closure in *Drosophila melanogaster*

Zsanett Lakatos, Ferenc Jankovics and Miklós Erdélyi

(Institute of Genetics, BRC HAS, Szeged)

Structure-function study of yeast RAD18.

Orsolya Frittmann, Vamsi Krishna Gali, Ildikó Unk

(Institute of Genetics, BRC HAS, Szeged)



Orthologs of the rabbit *Oct4*, *Sox2*, *Klf4* pluripotency genes

Flora Vajda, Viktoria Szabo, Diana Nagy, Anita Kaity-Szecsényi and Melinda Pirity

Institute of Genetics, BRC HAS, Szeged

Aims

Pathways governing pluripotency and consequent differentiation vary from species to species. Reprogramming of somatic cells to the pluripotent state has been achieved by combinations of transcription factors that belong to the core pluripotency circuitry: Oct4 (O), Sox2(S), Klf4(K), c-Myc(M) (OSKM). The aim of this study to compare the phylogenetic evolution of rabbit OSK pluripotency genes, with other relevant species in order to establish phylogenetic relationships among the rabbit and its closest relatives. Also, to test whether the rabbit OSK genes are closest to their human or rodent counterparts. Our hypothesis is that phylogenetic relationships may reveal differences and similarities in pathways governing pluripotency and differentiation among evolutionary distant species. A better understanding of complex phylogenetic relationships and investigating the functional shift between orthologues may facilitate the derivation, maintenance and growth of true (germline competent) rabbit ES cells which has not been achieved so far. Creating germ line competent rabbit ES cells is highly desirable as the rabbit is an ideal cell/animal model for certain human congenital disease (diabetes, cardiovascular disease). Also, the embryonic development of the rabbit mimics human development more appropriate then the mouse.

Materials and methods

Corresponding OSK sequences from multiple species were withdrawn from National Center for Biotechnology Information; NCBI. The rabbit OSK sequences were newly identified in our laboratory (Tancos Z., unpublished data). All sequences were confirmed by individual BLAST searches (NCBI) to determine whether the corresponding data matched well with the appropriate gene of *Oryctogalus c.* Homologue sequences from different species were analyzed based on a multiple sequence alignment of the proteins. The relative branch length of the neighbor-joining tree emphasize the degree of the relativeness between the branches. Initial alignment was conducted using a hierarchical method for multiple alignments implemented in the CLUSTAL-W tool in MEGA5 program. Bootstrap analysis used 1000 replicates. Gaps are rated as missing. Automatically aligned sequences were checked manually.

Results

Oct-4 (octamer-binding transcription factor 4) also known as POU5F1 (POU domain, class 5, transcription factor 1) is a protein, coded by the *POU5F1* gene in human and belongs to the POU transcription factor family.

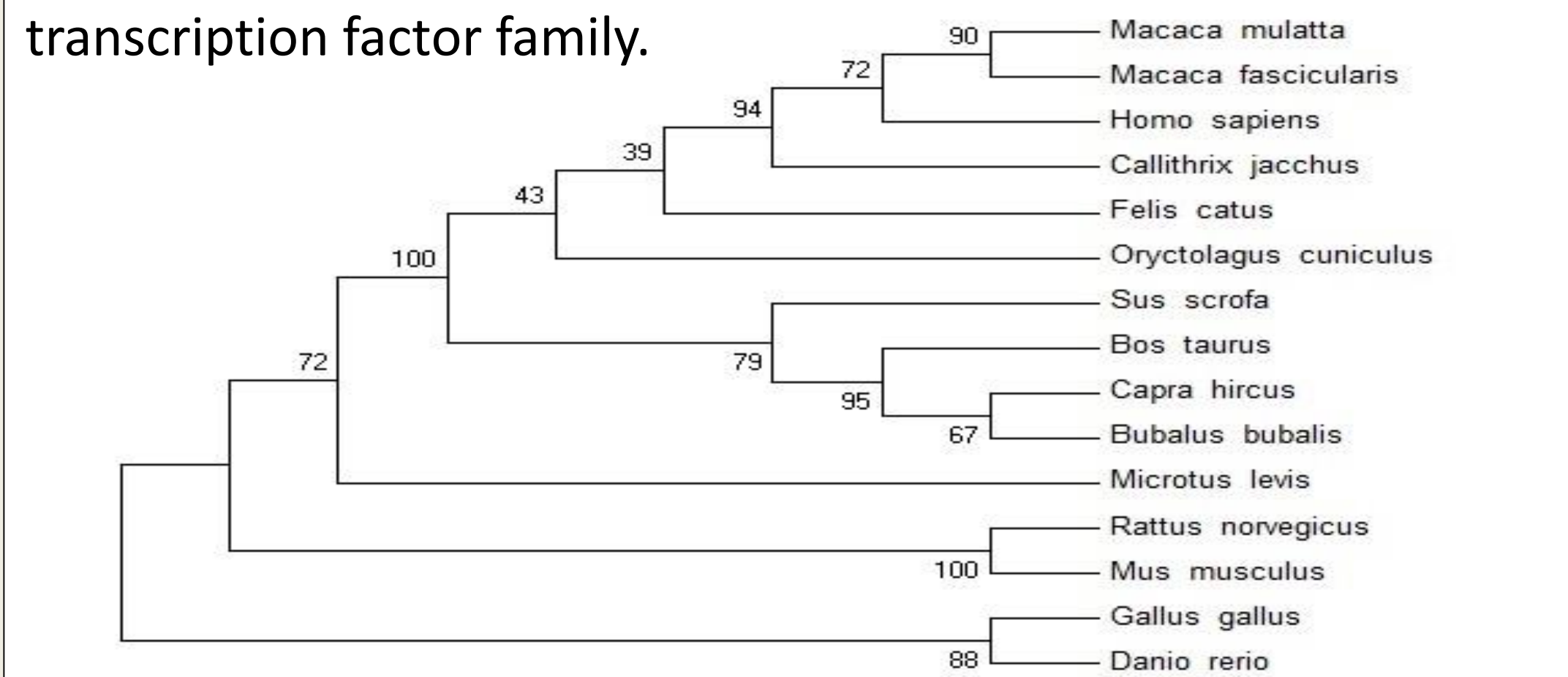


Figure 1.: Phylogenetic tree of the *Oct4* gene

The phylogenetic analyses showed that the *Oryctogalus c.* *Oct4* sequence is in a same cluster as the *Homo s.* and *Primate* sequences. Rodent *Oct4* sequences clustered in separate sub-branch. The amino acid sequences of *Gallus g.* and *Danio r.* are strictly different from other species; they represented the largest relative distance from *Oryctogalus c.* The amino acid sequence of *Bos t.* composes a different phylogenetic group with *Sus c.* and *Capra h.* sequences. These observations suggest that during evolution the *Homo s.* and *Oryctogalus c.* *Oct4* genes clustered together and that the function of *Oct4* in the pluripotency circuits are likely the most similar between these two species.

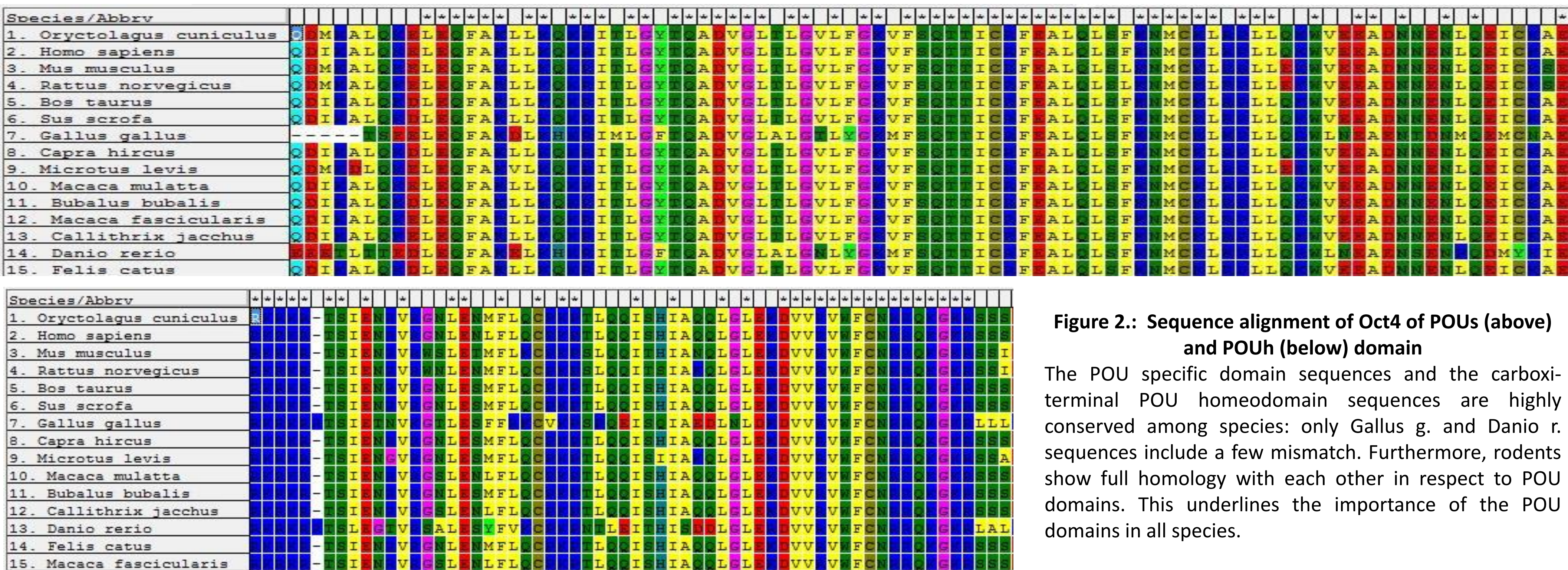


Figure 2.: Sequence alignment of Oct4 of POU domains (above) and POUh domains (below)

The POU specific domain sequences and the carboxy-terminal POU homeodomain sequences are highly conserved among species; only *Gallus g.* and *Danio r.* sequences include a few mismatch. Furthermore, rodents show full homology with each other in respect to POU domains. This underlines the importance of the POU domains in all species.

Sox-2 (sex-determining region Y-box 2) is a member of the Sox family of transcription factors and forms a trimeric complex with OCT4 on DNA in order to control the expression of a number of genes involved in embryonic development.

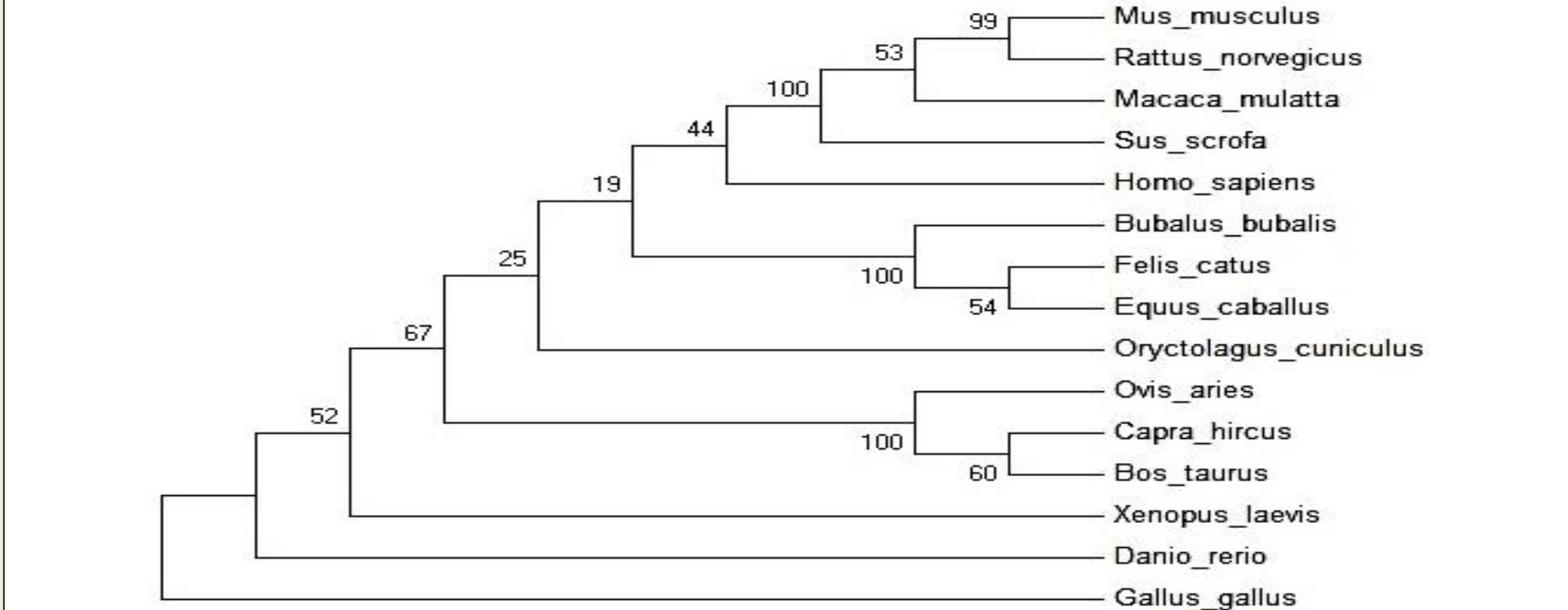


Figure 3.: Phylogenetic tree of Sox2

Mus m. and *Rattus n.* show close relationship and also near to *Homo s.* and *Rhesus m.* amino acid sequence. The closest relative of *Homo s.* is *Sus c.* *Bubalus b.*, *Felis c.*, and *Equus c.* (same cluster). *Bos t.*, *Capra h.* and *Ovis a.* form a different cluster. *Oryctogalus c.* is situated between these two major clusters. This shows that the phylogenetic evolution of *Oct4* and *Sox2* are different and not closely related to each other.

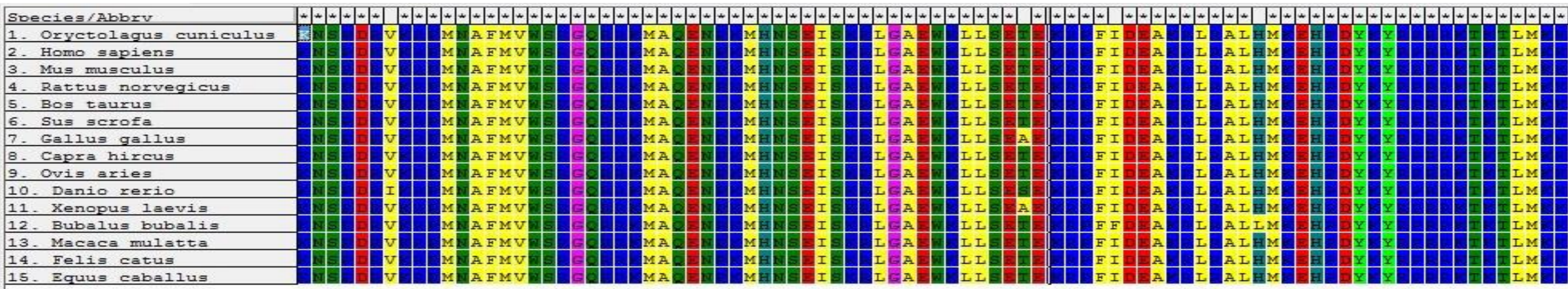


Figure 4.: Sequence alignment of the Sox2 HMG-box domain

The *Sox2* gene contains a functional High Mobility Group (HMG) domain, which can bind to the DNA or to other transactivation domains. *Mus m.* and *Rattus n.* HMG domains show the closest relationship in agreement with the full length *Sox2* protein alignments. *Homo s.* is located between the *Mus m.* and the *Oryctogalus c.* sequences, and it is very close to the *Sus c.*, *Bubalus b.* sequences which is in the same cluster as the *Equus c.* and the *Felis c.* *Sox2*. The *Bos t.* sequence represented as a subbranch with the *Ovis a.* and the *Capra h.* *Sox2*. This data suggests that the HMG functional domain evolution is not entirely recapitulates the full length *Sox2* protein phylogenetic tree.

The **Klf4** protein belongs to the family of Kruppel-like factor 4 proteins. It plays an important role in maintaining embryonic stem cells, and in preventing their differentiation. Klf4 also expressed in some differentiated cells not only in stem cell- in contrast with *Oct4*.

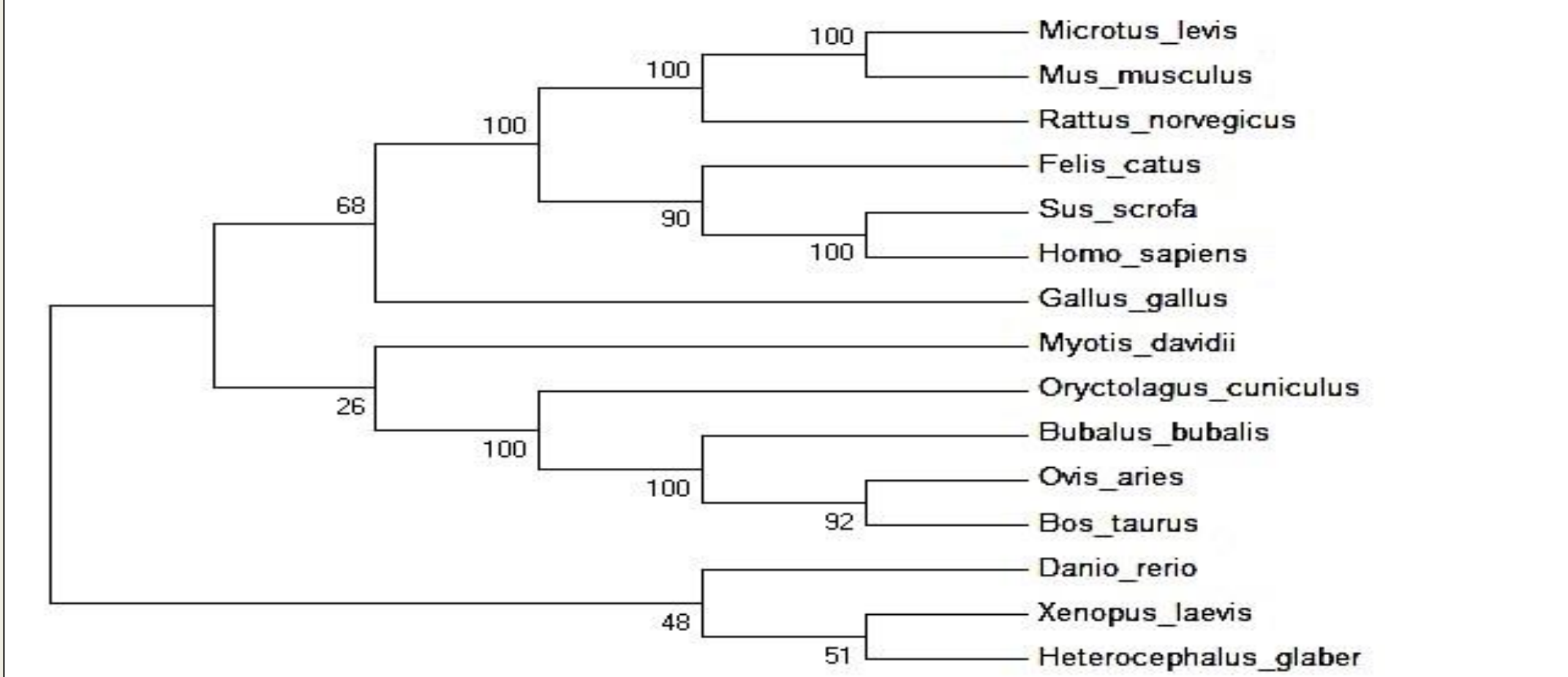


Figure 5.: Phylogenetic tree of Klf4

The full length *Klf4* sequence is organized into two major branches. The first branch contains the *Xenopus l.*, *Danio r.* and *Heterocephalus g.* *Klf4* sequences. The second branch sub-divided into two subbranches where *Oryctogalus c.* is relatively far from *Homo s.* suggesting there is probably less functional conservatism between the two species.

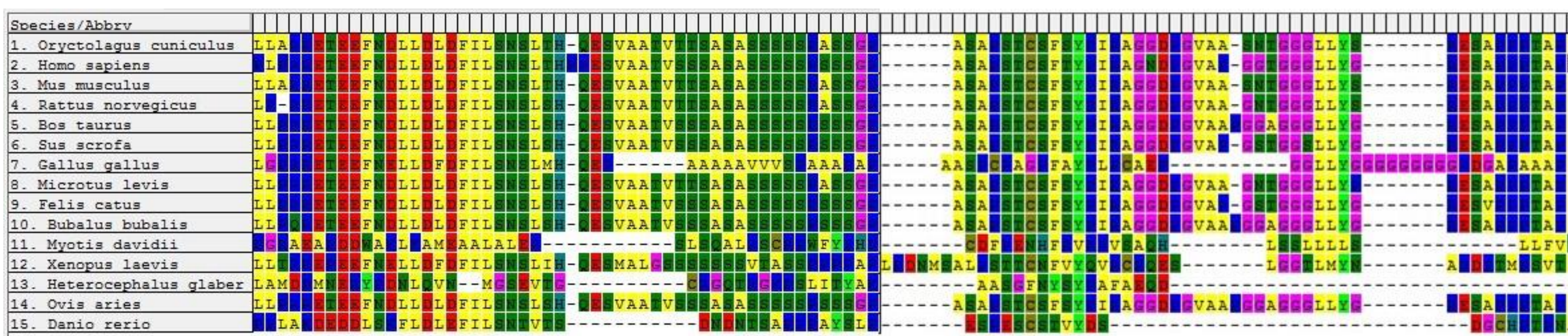


Figure 6.: Sequence alignment of the Klf4 activator and inhibitory domains and PEST sequences

The PEST sequence motif functions via the ubiquitin-mediated proteosomal degradation pathways. The PEST sequence of *Oryctogalus c.* and *Mus m.* sequences are highly homologues. *Rattus n.* sequence differ only in 1 amino acid from *Oryctogalus c.* and *Mus m.* Most like this function is strongly conserves among species (rodents and non-rodents).

Summary

The three pluripotency gene don't show the same evolution in phylogenetics among the examined species. Rabbit *Klf4* and *Sox2* seem to be more distant from their human counterparts then the rodent sequences, which suggests these pluripotency genes may play slightly different role among these species. However, during evolution, the protein domain of *Oryctogalus c.* and *Homo s.* show considerable homology to each other even in respect to the variable TAD domain module. Thus they seem to be functionally most related. We propose that besides the mouse, rabbit ES cells can also serve as ideal model system for human ES cell research. Furthermore, the most noted similarity between human and rabbit is in the *Oct4* protein sequences. Since *Oct4* is critical in all examined species for pluripotency - data presented in this poster underlines the importance of rabbit as an experimental model system of human development/pluripotency.

Acknowledgments:

This work was supported by NKTH-OTKA FP7“Mobility” HUMAN-MB08C-80205. Show