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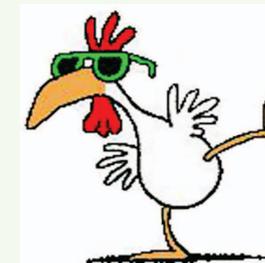
# Cloning of the chicken integrin $\alpha 4$ gene

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## Conclusion

We have shown that mRNA encoding the integrin  $\alpha 4$  protein is expressed in the chicken, and for the first time, the integrin  $\alpha 4$  cDNA has been cloned. The sequence reveals a polypeptide of 1021 amino acids with homology to the known mammalian integrin  $\alpha 4$  proteins. Structurally, chicken integrin  $\alpha 4$  resembles the mammalian counterparts; however, further investigations are necessary to determine whether chicken integrin  $\alpha 4$  is involved in leukocyte homing.



## Background

Integrins are heterodimeric membrane-spanning proteins composed of an  $\alpha$  and a  $\beta$  chain, and they function as cell adhesion receptors that recognize extracellular matrix ligands and cell-surface ligands. In mammals, integrin  $\alpha 4$  dimerizes with either integrin  $\beta 1$  or  $\beta 7$ . Both dimers can be expressed on leukocytes and are involved in leukocyte homing to different tissues, where the integrin dimers interact with distinct receptors on endothelial cells (see Fig. 1). This interaction contributes to the rolling, adhesion, and extravasation of leukocytes. In chickens, the existence of the integrin  $\alpha 4$  gene has been predicted from computational analysis of the genomic sequence, and the finding of several expressed sequence tags supports the notion that the gene is transcribed into mRNA. However, the gene has not yet been cloned.

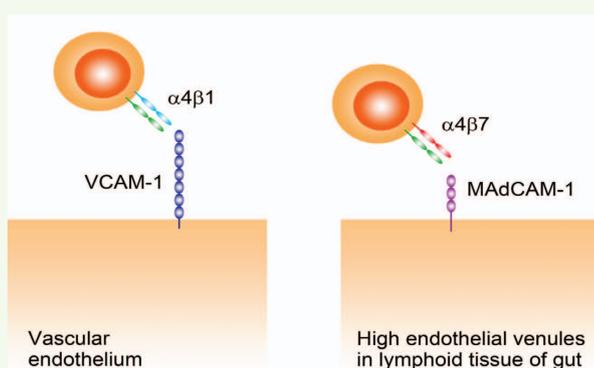


Figure 1. In mammals, integrin  $\alpha 4\beta 1$  dimers bind to Vascular Cell Adhesion Molecule-1 (VCAM-1), which is expressed on vascular endothelium throughout the circulatory system. Integrin  $\alpha 4\beta 7$  dimers bind to Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1), which is restricted to endothelial cells in lymphoid tissues of the gut, such as Peyer's patches.

## Discussion

In mammals, integrin  $\alpha 4$  forms dimers with integrin  $\beta 1$  or  $\beta 7$ . Since the predicted protein sequence and structure of chicken integrin  $\alpha 4$  is similar to the known mammalian orthologs, one could assume similar functional properties. However, searches in the chicken genomic database only reveal the integrin  $\beta 1$  gene and not the integrin  $\beta 7$  gene. The integrin  $\alpha 4\beta 7$  dimer is responsible for leukocyte homing to lymphoid tissue of the gut. What the lack of integrin  $\beta 7$  in the chicken means for leukocyte homing is not known. Perhaps another member of the integrin  $\beta$  family takes over or perhaps other homing mechanisms are in play.

## Results

### The chicken integrin $\alpha 4$ gene

According to the Ensembl database, the chicken integrin  $\alpha 4$  gene is located on chromosome 7 and consists of 28 exons (Fig. 2). The gene spans 32.3 kb.

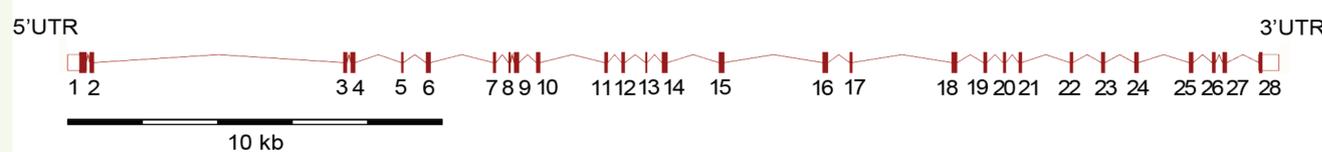


Figure 2. Genomic organization of the gene encoding chicken integrin  $\alpha 4$ . The gene consists of 28 exons that span 32.3 kb on chromosome 7. Data was obtained from Ensembl Genome Browser (<http://www.ensembl.org/index.html>).

### Cloning of integrin $\alpha 4$ cDNA

Total RNA was purified from chicken peripheral blood leukocytes and converted into cDNA, which was used as template in PCR amplification. The integrin  $\alpha 4$  cDNA was successfully cloned using primers located in the 5' and 3' untranslated regions. The cDNA sequence contains 3755 nucleotides including an open reading frame encoding a polypeptide of 1021 amino acids. Two nucleotides in the open reading frame (T321 and A567) differ from the database sequence (Ensembl transcript ENSGALT0000001460, UniProtKB 7Q9PSD7\_CHICK), but in both cases the nucleotide substitution does not change the encoded amino acid. The amino acid sequence is shown in Fig. 3. The calculated molecular mass is 114 kDa.

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MRS CGRAARW AAP LLLLWQC LPTARTYNVD TRHPLLFRGD NGTFFGYSVL LMGHGEERWL 60
VVGAPQASWA ANSSVISPGA IFRCRIGSNP RGSCEQLQLG HPSGEYCGKT CMEERDYQWL 120
GVLSLRQPRE NGSFVACGHR WKNIFYIKND HKLPHGICFA VSSDFRTELS RRICPCYKDH 180
VRKFGENHGS CQAGMSSFYI GDLLIMGAPG SYWTGVSFV YNTTINTIHA YTHSNQVKF 240
GSYLGYSVGA GHFLTPSSSTE VIGGAPQQEQ TGKAFIFSID EHLNVLFEVK GKKLGSYFGA 300
SVCVAVLNSD GLSDDLVGAP MESTIREEGR VVYVINSQSK AEMVELDIEL SSSDYSYARF 360
GESITNLGDI DNDGFEDVAI GAPQEDDLKG AIYIYNGRED GITPSFSQRI PAQVSTSL 420
MFGQSIASGI DADNNGYQDI AVGAFLSDSA VVLRTPVII VEAFLKHKPS INRTNLN 480
NDQPAICVNL QICFNVTGQG VPDNTEFYN LSVDVKRRVD TQARFYFSAN GTSETTSGSI 540
KINRKI IACK GHAFMRKDV RDILTVPVHE ASYHLGQQL QKRDNQELSA LPPVLQRRKE 600
KDIKSKFVF ARICSQENCS ADLRVSGKVA FPKPHDKMY LVVSTKTL LNLISLH 660
DAYETVLHIQ FPKGLYFIRV PDLEEKIHC EVLKDIDHAV KLECSVGYLY VDQNSKLDLS 720
FPLDTSSFT R AEDDLNLIIN VSCKNENEN LLDNMVTVAV PLKYETELNT HGFVTPPSFV 780
YGTNEEASV MCMENINFT FHLINAGPSM APNINLEMI PNAPPHDFK LFNVMIDKTT 840
VGECSYNEYR RNCNAPEKTE NILKDVVTF SKPAKRMVC MKNDSLCLQI HCKLGNMENG 900
KEATTIQLHLE ATPALLEMDD ASTLKFVRA TASPENAKV IELQKDKQVA YVYLEGVHQ 960
KPKYHVTVLI IGIGLIAGIT LFLLLSLLL KIGFFKRQYK PIPQDMNRR SWSFTSGNKD 1020
D
    
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Figure 3. Amino acid sequence of chicken integrin  $\alpha 4$ .

Table I. Sequence identity in percent between integrin  $\alpha 4$  proteins of different species.

Identity (%)	Chicken	Human	Chimpanzee	Mouse	Rat	Dog	Cow
Chicken	-	60.7	60.7	60.4	59.8	52.3	61.0
Human	60.7	-	99.4	84.8	84.1	74.1	85.6
Chimpanzee	60.7	99.4	-	85.0	84.2	74.3	85.8
Mouse	60.4	84.8	85.0	-	93.8	70.5	80.4
Rat	59.8	84.1	84.2	93.8	-	69.8	79.8
Dog	52.3	74.1	74.3	70.5	69.8	-	71.7
Cow	61.0	85.6	85.8	80.4	79.8	71.7	-

### Sequence comparison

The amino acid sequence of chicken integrin  $\alpha 4$  is similar to integrin  $\alpha 4$  sequences in other species. Multiple alignments using the ClustalW2 alignment tool at <http://www.ebi.ac.uk> demonstrate a sequence identity of 52-61% between chicken integrin  $\alpha 4$  and the integrin  $\alpha 4$  of several mammals (Table I). Between the mammalian species, the sequence identity is higher (70-99%).

### Protein structure

The integrin  $\alpha 4$  protein has a large extracellular region consisting of aa 1-967 (of which the first 26 aa constitute a signal peptide), and this is followed by a short transmembrane region (aa 968-990) and a cytoplasmic tail (aa 991-1021). As shown in Fig. 4, the domain architecture is similar to that of integrin  $\alpha 4$  in mammals, and the extracellular region includes the so-called  $\beta$ -propellor, thigh, calf1, and calf2 domains.



Figure 4. Protein structure of chicken integrin  $\alpha 4$  as predicted by InterProScan Sequence Search (<http://www.ebi.ac.uk/Tools/InterProScan/>). SIG: signal peptide, TM: transmembrane region, CYT: cytoplasmic tail.