# Alternative splicing of delta-like 1 homolog (*DLK1*) in the pig and human

Jeffrey A. Deiuliis [a, b], Bing Li [a], Pasha A. Lyvers-Peffer [a], Steven J. Moeller [a], Kichoon Lee [a, b,*]
[a]Department of Animal Sciences and [b] The Ohio State University Interdisciplinary Human Nutrition Program, The Ohio State University, 2029 Fyffe Rd, Columbus, OH 43210

## Abstract

Delta-like homolog 1 (*DLK1*), a paternally imprinted gene with several alternative splicing isoforms, is an important regulator of fetal and postnatal development. We report here the sequence of porcine *DLK1* (*pDLK1*) and examine the expression and alternative splicing isoforms in the pig (*Sus scrofa*) and human. *DLK1-A* was the sole isoform identified in human tissues and has been shown to be present in mouse and cattle. Surprisingly, *DLK1-A* was undetected in various tissues from fetal and postnatal pigs. Instead, *DLK1-C2* was the most abundant isoform while *DLK1-B* was expressed to a lesser extent. In fractionated adipose tissue, *pDLK1* was most highly expressed in the stromal-vascular cell fraction. In addition, total *pDLK1* was highly expressed in fetal adipose tissue but dramatically decreased postnatally. Our data suggests that expression of *DLK1-B* and *-C2* isoforms is sufficient for normal pig development. Furthermore, human and pig samples showed no alterations in species-specific splicing, but expression levels decreased with age suggesting that regulation of expression, not splicing, is the most likely mechanism controlling the biological function of *DLK1*.

## Introduction

*DLK1* biology is important to the agricultural research community because the desirable callipyge phenotype in sheep has been shown to be due to the overexpression of *DLK1*. *DLK1* overexpression in transgenic mice presented a callipygian phenotype with increase muscle development and reduced adipocity[1]. Several Dlk1 protein isoforms have been reported in various species which have been shown to be the product of alternative splicing (AS) of primary transcript mRNA. In the mouse, six AS isoforms have been identified[2,3]. It is debated whether the cow has two AS isoforms[4], T. et al. 2003) or three[5]. *DLK1* AS in humans has not been thoroughly examined previously. The roles of *mDLK1* isoforms (named A-D) have been studied during mouse adipocyte differentiation. *DLK1-A* and *-B* transcripts code for protein products that can be cleaved to 50 and 25kDa soluble polypeptides[3]. The 50kDa protein has been shown to prevent adipocyte differentiation in culture[6;7]. Short form Dlk proteins (Dlk1-C, -C2, -D, -D2) contain only one proteolytic site producing the 25 kDa soluble protein which did not prevent preadipocyte differentiation *in vitro*[7]. We reported here the unique alternative splicing pattern of the *DLK1* gene in the pig and human and compared the sequences of splicing junction sites in several species. Our investigation includes tissue distribution of splicing isoforms, development alterations in expression level, and splicing with porcine adipose tissue development.

## Materials and Methods

- *PCR amplification of DLK1 and Cloning*
- *RNA isolation and preparation of cDNA*
- *Quantitative-real-time PCR detection of total gene expression and semi-quantitative detection of alternative splicing isoforms in pig and human.*
- *Porcine stromal-vascular and fat cell fractionation*
- *Bioinformatics, sequence analysis, and statistical analysis*

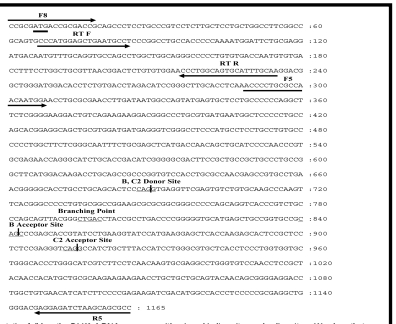


Fig 1. The putative full length *pDLK1-A* DNA sequence with primer binding sites and splice sites. We show that the pig expresses two splice isoforms of *DLK1*. The B and C2 isoform share a 5' splice site and have different 3' acceptor splice sites. The B acceptor site is an incomplete match to the classical splicing consensus sequence but the C2 isoform is a complete match. The branching site is located approximately 50 bp from the B acceptor site and 120 bp from the C2 acceptor site.

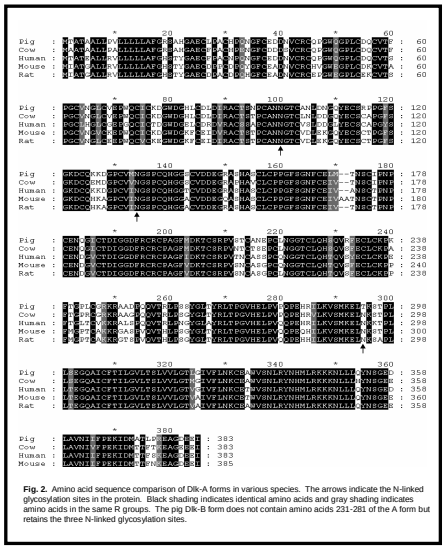

**Fig. 2.** Amino acid sequence comparison of Dlk-A forms in various species. The arrows indicate the N-linked glycosylation sites in the protein. Black shading indicates identical amino acids and gray shading indicates amino acids in the same R groups. The pig Dlk-B form does not contain amino acids 231-281 of the A form but retains the three N-linked glycosylation sites.

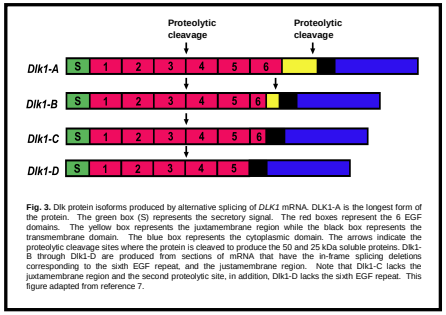


**Fig. 3.** Dlk protein isoforms produced by alternative splicing of *DLK1* mRNA. DLK1-A is the longest form of the protein. The green box (S) represents the secretory signal. The red boxes represent the 6 EGF domains. The yellow box represents the juxtamembrane region while the black box represents the transmembrane domain. The blue box represents the cytoplasmic domain. The arrows indicate the proteolytic cleavage sites where the protein is cleaved to produce the 50 and 25 kDa soluble proteins. Dlk1-B through Dlk1-D are produced from sections of mRNA that have the in-frame splicing deletions corresponding to the sixth EGF repeat, and the justamembrane region. Note that Dlk1-C lacks the juxtamembrane region and the second proteolytic site, in addition, Dlk1-D lacks the sixth EGF repeat. This figure adapted from reference 7.

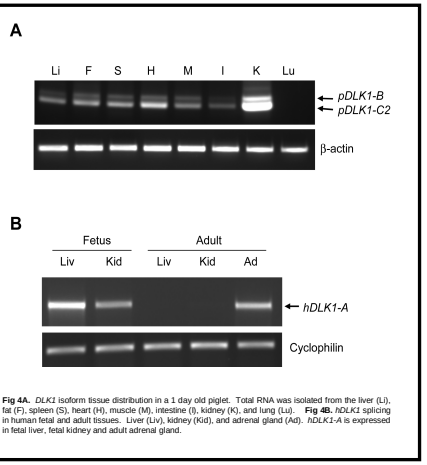


**Fig 4A.** *DLK1* isoform tissue distribution in a 1 day old piglet. Total RNA was isolated from the liver (Li), fat (F), spleen (S), heart (H), muscle (M), intestine (I), kidney (K), and lung (Lu). **Fig 4B.** *hDLK1* splicing in human fetal and adult tissues. Liver (Liv), kidney (Kid), and adrenal gland (Ad). *hDLK1-A* is expressed in fetal liver, fetal kidney and adult adrenal gland.

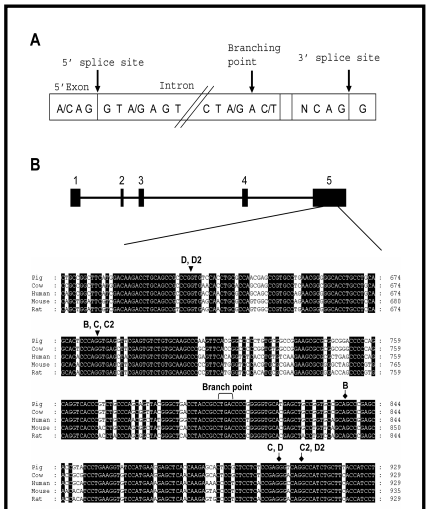


**Fig. 5A.** Classical model of primary pre-mRNA splicing. This model is based on the consensus sequences identified as the exon-intron junction splice site in the removal of intron sequences from pre-mRNA. This diagram is an adaptation from Molecular Cell Biology, 5th ed. Lodish, 2003 based on the research of Padgett 1986, Keller and Noon 1984. **Fig 5B.** *DLK1* DNA sequence comparison across species. The known 5' (arrow) and 3' (diamond) splice site in the pig, cow (GenBank accession no. ***AF181466***), human (GenBank accession no. ***BC007741***), mouse (GenBank accession no. ***BC052159***), and rat (GenBank accession no. ***NM_053744***) are illustrated. Black shading indicates identical nucleotides.

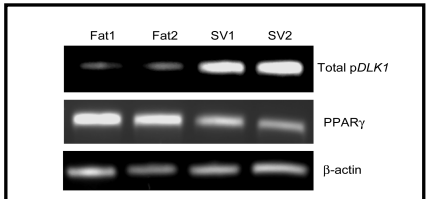


**Fig. 6.** Relative *DLK1* gene expression in the adipocyte and stromal-vascular (SV) fraction of digested tissues from two 12 day old piglets. Total *DLK1* expression is shown in addition to PPARg expression, an adipocyte marker gene, both of which are normalized to b-actin.

**Table 1. Primers**

| Name | Sequence |
|---|---|
| pDLK F8 | 5'-CCGCGATGACCGCGACCG-3' |
| pDLK F5 | 5'-ACCCCTGCGCCAACAATGGA-3' |
| pDLK R5 | 5'-GGCGCTGCTTAGATCTCCTC-3' |
| pDLK RT F | 5'-CCCATGGAGCTGAATGCCT-3' |
| pDLK RT R | 5'-TTGCAAATGCACTGCCAGGG-3' |
| pPPARg F | 5'-CACAGGCCGAGAAGGAGAAG-3' |
| pPPARg R | 5'-ATCTCCTGCACAGCCTCCAC-3' |
| pFABP4 F | 5'-GGCTTTGCTACCAGGAAAGT-3' |
| pFABP4 R | 5'-CGCAGTGACACCATTCATGAC-3' |
| pb-actin F | 5'-CGTGGGCCGCCCTAGGCACCA-3' |
| pb-actin R | 5'-TTGGCCTTAGGGTTCAGGGGGG-3' |
| pCyclophilin F | 5'-GGATAATTTTGTGGCCTTGGC-3' |
| pCyclophilin R | 5'-ACTGGGAGCCATTGGTGTCT-3' |
| hDLK F | 5'-GATGTTCGGGCCTGCTCCT-3' |
| hDLK R | 5'-ACCACCAGGCTGGTGAGCA-3' |
| hCyclophilin F | 5'-CTCCTTTGAGCTGTTTGCAG-3' |
| hCyclophilin R | 5'-CACCACATGCTTGCCATCC-3' |

## Acknowledgements

This work was supported by an Ohio Agriculture Research and Development Center (OARDC) seed grant.

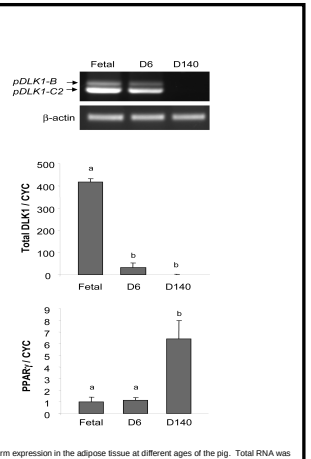


Fig.6 A) *pDLK1* isoform expression in the adipose tissue at different ages of the pig. Total RNA was isolated from 70-day old fetal, 6-day old and 140-day old pig adipose tissues. PCR for *pDLK1* splicing isoforms using the pDLK F5/R5 primer set is shown in an agarose gel. B and C) Quantitative real time RT-PCR for total *pDLK1* and PPARg expression in pig adipose tissue at three different ages. Bar charts represent data from at least three animals per group performed in duplicate and are corrected for cyclophilin. Data reported as means ± S.E.M. The levels of total *DLK1* expression at day 6 and day 140 were significantly lower than that of the fetus (ANOVA, $p < 0.01$). The levels of PPARg expression in pig adipose tissue at day 140 were significantly higher than those at fetus and day 6 (ANOVA, $p < 0.001$).

## Conclusions and Discussion

We report here for the first time the pig *DLK1* sequence and show that the gene contains conserved splicing consensus sequences in the pig, cow, human, mouse, and rat. *DLK1* is known to have a variety of splice variants; however, the C2 isoform is dominant in all tissues and stages of development of the pig. The B isoform is present but in lesser amounts. In the human the A isoform is expressed exclusively, regardless of tissue or age. *DLK1* plays an important role in a variety of biological processes in multiple tissues and organ systems; however, in the human and pig the splicing pattern is consistently regardless of tissue or age. In addition, we compared this splicing pattern to that of the human fetus and adult and showed that there were species-specific splicing differences despite very comparable splice recognition sites suggesting species-specific alternative splicing mechanisms of *DLK1*. The splicing pattern of *DLK1* in the human and pig contrast strongly not only with each other but also with both the mouse and cow. The biological and evolutionary pressures leading to species-specific splicing of *DLK-1* are unknown and remain to be studied. Furthermore, *DLK1* is highly expressed in embryonic tissues in human and pigs, as shown in our studies, suggesting an important role for *DLK1* in fetal development.

## References

1. E. Davis et al., *Curr.Biol.* 14, 1858-1862 (2004).
2. C. M. Smas, D. Green, H. S. Sul, *Biochemistry* 33, 9257-9265 (1994).
3. C. M. Smas, L. Chen, H. S. Sul, *Mol.Cell Biol.* 17, 977-988 (1997).
4. T. Vuocolo, R. Pearson, P. Campbell, R. L. Tellam, *Comp Biochem.Physiol B Biochem.Mol.Biol.* 134, 315-333 (2003).
5. S. C. Fahrenkrug, B. A. Freking, T. P. Smith, *Biochem.Biophys.Res.Commun.* 264, 662-667 (1999).
6. H. Makarenkova, H. Sugiura, K. Yamagata, G. Owens, *Biochim.Biophys.Acta* 1681, 150-156 (2005).
7. B. Mei, L. Zhao, L. Chen, H. S. Sul, *Biochem.J.* 364, 137-144 (2002).