CHAPTER IV

DISCUSSION

In the present study the cDNA sequences of the vinculin gene was screened for polymorphic sites in 5 pig breeds. All point mutations that have been found are silent mutations. Comparision of vinculin cDNA sequences cloned from the 5 breeds identified no differences in amino acid composition within gene. The detection of the differential expression of the vinculin gene by the use of DD-RT-PCR between F2 high, F2 low and GL high, GL low performance animals which has been described by Ponsuksili *et al.* (1999,2000b) indicates that it may affect the trait eye muscle area, but this effect is not likely to be due to a mutation in the coding region of the vinculin gene. This is important because it indicates that although primary sequences of the transcribed region of this gene were identical among the individuals representing diverse populations, the quantities of mRNA were different. These differences could be caused by regulation occurring in the rate of transcription and mRNA stability. This provides evidence for a basis of genetic variation that is observed at the phenotypic level. The predicted protein sequence for human vinculin (Moiseyeva *et al.*,1993) and the amino acid residues at the point mutations which have been observed in this study are identicaly indicating that these regions are conserved. Multiple sequence alignment of chicken, human, mouse, *C.elegan* revealed differences of the amino acid sequences between these species. Vinculin expression

was previously shown to be regulated at the level of transcription in growth-activated cells (Bellas *et al.*,1991). The differential expression of vinculin which has been detected by the DD-RT-PCR is likely to be due to genetic variation that occurs at the promoter sequence or transcription factor binding sites affecting the transcription level or amount of mRNA. The number of potential points of gene regulation in living cells is virtually infinite, although a majority of the mRNA is found in polysome complexes, the formation of a nontranslatable mRNA : protein complex in the cytoplasm could potentailly be one possible translation regulation control point (Farrell.,1993). Therefore, the differential expression may be caused by a of a variety of factors influencing the prevalence of mature mRNA in the cytoplasm, transcriptional efficiency, transcription rate, splicing efficiency of precursor hnRNA molecules, nucleocytoplasmic transport and their translatability. In the case of the *c-fos* gene, the transcriptional response to platelet-derived growth factor, nerve growth factor, epidermal growth factor and insulin in a variety of cell type has been shown to be dependent on a 20 bp nucleotide sequence upstream of the transcription start site and termed a serum response element (SRF). This element is found in the promoters of a number of other early response genes (reviewed in the Moiseyeva *et al.*, 1993).

The result of physical mapping using the RH-panel confirms the previous chromosomal assignment based on analyses of a somatic hybrid panel done by Ponsuksili et al (2000). The porcine vinculin gene contains 1677 amino acid residues. The structure of porcine vinculin is similar to that of the human vinculin, with N-terminal domain, 3 central repeat, proline-rich region and the C-terminal. Nematode vinculin contains 2 central repeats and has a lower molecular weight compared to human and porcine vinculin. In chick vinculin, amino acid residues 167-207 were

lossed. This sequence is essential for the activity of the talin binding domain and the ability of this region of the protein to localize to cell-matrix junction where expressed in *Cos* cell.

The recent findings show that cultured mammalian vinculin-decicient cells from proper integrin-based focal adhesions and indicate that vinculin gene maybe not vitally important in mammals (Alatortsev *et al.*,1997). Furthermore, Alatortsev *et al.* (1997) reported that the flies *Drosophila melanogaster* carrying disrupted vinculin gene as a result of the X chromosome inversion are viable and fertile suggesting that the vinculin gene is not of vial importance in the flies. Although the dual nature of the nematode *Caenornabditis elegans* dense body may be similar to the actin-membrane attachments in nascent vertebrate myofibrils of developing striated muscle, which also have the characteristics of both focal adhesion and z-discs, the two mutations on the nenatode vinculin gene at the position st385 which response to prevents splicing of the fourth intron and at position sst555 which change generated an in-frame UAG amber stop codon leading to trancation of the protein at amino acid residue 565. It seems that both mutation lead to arrest development as L1 larvae, embrionic elongation was interupted at the twofold length so that the mutants were shorter than wild type animals at the same stage, the phenotype consistent with the idea that vinculin is essentail for muscle function in nematode (Barstead *et al.*, 1991). However, given the evidence for limited alternative splicing of the vinculin gene which seems likely that much of the molecular heteroginity in vinculin is due to post-translational modification (Moiseyeva *et al.*,1992). It has been reported that increased levels of phosphorylated vinculin were detected after activation by growth factors and it was shown that a tissue-spesific distribution of phosphorylated vinculin isoforms

occur at discrete stage in vertebrate development. Reversible phosphorylation of vinculin is a potential candidate for such a master regulatory step and indeed, vinculin has been described as a substrate of PKC. During the junctional sealing induced by extracellular calcium, vinculin phosphorylated by PKC is a critical step in the correct assembly of the epithelial junction complex (Perez-Mereno *et al.*, 1998). Refer to the structure of vinculin which coresponding to talin, Frenette *et al.* (1998) reported that talin is a key structure link between the cytoskeleton and cell membrane regulated the developing in adult muscle, thereby, the expression of vinculin may be due to talin. Bershadsky *et al.* (1995) have studied the expression of vinculin in cell by treated with drug. They have found the increased level of assembled actin resulted in elevated actin synthesis and RNA content, these studies suggest the existence of an autoregulatory path way for the expression of actin and other microfilament-associated-proteins, including vinculin, which is linked to the state of actin polymerization in the cell. The state of actin assembly regulates actin and vinculin expression by feedback loop.

A major objective of applying molecular techniques to the improvement of animals is to identify and clone major genes influencing economically important traits. Identification of such genes relies largely on conventional techniques as well as creative methods for exon identification. The candidate gene approach is one commonly used method. However, this method only permits screening of know molecules on an individual basis and depends on assumptions made by the researchers and it identifies differences in gene expression (Matteri *et al.*,1996). The Human Genome Research Institute (HGIS) is estimated that there are on average four single nucleotide differences called polymorphisms (SNPs) per individual messenger RNA

of any two people (Human Genome Sciences., 2000). To apply this method to animal improvement, properly defined populations, individuals, tissues, and development stages must be considered, particularly in the case of genes regulated during development. As vinculin shares considerable structural similarity with alpha-catenins which are involved in adherents-type cell-cell junctions, taking into account a similarity in structure and site of location, alpha-catenin could be considered as a candidate molecule substituting for vinculin (Janssens *et al.*, 1999). In vertebrate muscle, meta-vinculin is coexpressed with vinculin (Riediger *et al.*, 1998), the immunofluorescence with antibodies discrimination between meta-vinculin and vinculin revealed that they are colocalize in the same adhesion site, i.e. in costameres and in the membrane-apposed attachment plaques of smooth muscle cell (Belkin *et al.*, 1988). Vertebrate myogenesis involves a number of steps controlled by a variety of signals and is ultimately orchestrated by regulatory events at the gene level in muscle cells, unlike the other cell types in the body, the muscle fiber is multinuclear and there is evidence that transcriptional activity varies between nuclei within the muscle fiber (Gullberg *et al.*, 1998). Taken together, it is conceivable that this broadly expression of vinculin would be the cause of the phenotypic variation in such animal. It could, therefore, have an importance in different express of mRNA level including how vinculin gene is correspond to the the trait EMA.

In the recent studies of the genomic scan to detect quantitative trait loci in swine, there were a few researches identified body composition traits, fat deposition, eye-muscle area, mucling and carcass length which are the important traits in the price of market hogs. Considerable differences have been reported for these traits between Large White and European wild boar (Anderson *et al*,1998) and Meishan –White

composite boars (Rohrer and Keele.,1998a,b). The intercross between Meishan-White composite boars found that three genomic regions were significant for measures of subcutaneous fat composition over the back on chromosome 1 and X for loin eye area, Boston butt adjusted to a constant live (TWPCWT) and carcass (TWPLWT) weight, chromosome 7 for hot carcass weight and carcass length. The QTL on chromosome 8 and 9 displayed suggestive linkage for leaf fat and the regions presented evidence of QTL for backfat are on chromosomes 5, 10, 13 and 14. Meisan alleles produced fatter pigs for all loci except on chromosome 7 and 10. For the intercross between Large White-European wild boar, indicated the significant QTL effects were found for percentage lean meat and percentage lean meat plus bone in various cuts, propotion of bone in relation to lean meat in ham, muscle area and carcass length on chromosomes 3, 4 and 8. Gene action for most QTL was largely additive. The average proportion of wild boar alleles across the genome had highly significant effect on reflectance and drip loss. The region on SSC 14 had suggestive evidence for QTL affecting loin eye area, TWPCWT, TWPLWT, average backfat, fat over first rib and last lumbar vertebra. Moreover, genetic marker on the traits eye-muscle area and length of carcass have found at position EagtTaacI on chromosome 4 in the Berlin-Bonn resource population by the use of Amplified Fragment Length Polymorphism (AFLP) (Murani *et al.*, 2000).

The polymorphic sites that were detected in this study are useful as DNA-markers that might be closely linked to polymorphism affecting carcass traits. Such polymorphisms may exist in the regions of the vinculin gene that are involved in regulation of gene expression, three PCR-RFLPs have been established that can be used to genotype at the polymorphic sites within the vinculin gene. The PCR-RFLP

of the SNP at position 4197 has the advantage to detect a non-variable cutting site for the restriction endonuclease MboI, that is useful as an internal control of the success of the restriction reaction.

Thus, many candidate genes for particular trait must be screened to identify a few QTL and different candidate gene requires large scale identification of genomic sequences and polymorphic sites with in known candidate genes and definition of intragenic haplotypes for maximum power (Soller., 1998). One of the potential major benefits of selection based upon marker information is that maker genotypes can be determine from sample that can easily collected from an animal as soon as it is born. Thus, marker information can be used to predict an animal's genotype for some important genes and provide a prediction of its likely phenotypic performance. This prediction can be made even in animals which will never express the trait.