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Host genetic response to PRRS virus

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Introduction

The breeding of domesticated animals used for food production has been directed primarily at increasing performance as measured by production, growth rate, reproductive success, and carcass quality. Although susceptibility to several infectious diseases is known to have a genetic component, only recently is emphasis being placed on the selection of animals based on disease resistance. Instead, livestock production systems have employed extensive and expensive vaccination, antibiotic, and chemoprophylactic methods to maintain herd health. Such practices can significantly cut in to producer profit margins, with the costs of disease estimated at 10–20% of total production values (Muller and Brem, 1991). Moreover, vaccines for several important veterinary pathogens are presently unavailable or of limited efficacy. Most importantly, there is a growing public interest for producing "natural" food supplies. As a result, identifying genes that impact both general and disease-specific immunity in pigs is of tremendous value. To date, most investigations of host-pathogen interactions in pigs have utilized a candidate gene approach, subsequent to information gleaned from rodent model systems.

Porcine reproductive and respiratory syndrome (PRRS) disease causes significant economic loss to the pig industry. PRRS is associated with interstitial pneumonia and failure to thrive in post-weaning piglets, and abortion in sows. The causative agent is PRRS virus (PRRSV, Collins et al., 1992), a 15kb positive-strand RNA virus in the Arteriviridae family. Replication of most arteriviruses, including PRRSV (Molitor et al., 1996), is largely limited to macrophages. Alveolar macrophages are the most susceptible pig cell type in which PRRSV replicates (Pol et al., 1992). A frustrating aspect of PRRS is the great individual variability in disease severity observed on infected farms, even for farms harboring a single PRRSV strain. Variation in severity of clinical disease among infected pigs in a herd and among herds suggests that genetic differences may exist for resistance or susceptibility. Indeed, pig breeds display differential susceptibility to certain PRRSV-induced lesions (Halbur et al., 1997), suggesting that allelic variation in disease response genes may exist for PRRSV. Thus, our efforts have focussed on understanding how the infected macrophage responds to the virus (Zhang et al., 1999). It is anticipated that the PRRSV-response ESTs we have identified include key host proteins that participate in cell-virus interactions. Subsequent identification of functional polymorphisms provide for biochemical delineation of host genetic factors that impact disease severity, including the tendency for persistent infection. Finally, these interfaces between host cells and the virus are also potential targets for chemotherapeutic clearance of PRRSV and perhaps other viruses.

Molecular responses to infectious agents

Major genes that control humoral and cellular immune responses to a variety of antigens map to a large chromosomal region (3–4 Mb) known as the major histocompatability complex (MHC). MHC haplotypes are associated with resistance or susceptibility to several diseases (Schook et al., 1996). The primary function of MHC molecules is to present endogenous and exogenous antigens to T lymphocytes, with consequent effects on lymphocyte proliferation, antibody production, and cell-mediated cytolysis. Studies in humans and mice have shown that polymorphisms at several MHC loci are associated with specific immune and autoimmune parameters. However, no association between MHC alleles and severity of PRRSV disease has been reported. Clearly, products encoded within the MHC contribute to host responses to infectious challenge but cannot account for the full development of immunocompetent phenotypes.

Remarkable progress has been made in the identification and characterization of genes responsible for hereditary diseases in humans. However, the diseases identified are single locus traits or multigenic traits where one gene contributes disproportionately to disease. With few exceptions, resistance to infectious disease is more complex and often involves various combinations of genetic and environmental factors. In a few rare cases, genetic resistance to a pathogen is controlled by a single major locus, such as resistance to *Escherichia coli* K88 in neonatal pigs resulting from the absence of the necessary receptor (Sellwood et al., 1975). An additional example is described in mice where the antiviral state against influenza A and B viruses is controlled by the autosomal domi-

nant Mx1+ allele (Muller and Brem, 1991). However, single locus resistance is usually restricted to a single pathogen, does not predict overall immunocompetence, and is not necessarily transferable across species. For example, Mx1 proteins in humans contribute to activities against influenza virus, but are not sufficient in and of themselves (Horisberger, 1995). Similarly, Mx1 expression is induced during PRRSV infection, but does not completely block virus replication or accumulation of viral mRNAs (Zhang*et al.*, 1999). The relevant loci that show altered expression during PRRSV infection are only now being elucidated.

Macrophage-pathogen interactions

It is important to remember that once a virus invades a host cell, the battle often becomes physiological and biochemical, as well as immunological. Viruses usurp host biological processes for their own benefit, and they have developed an array of tools to accomplish this. In return, the host cell manipulates its own gene expression in order to maintain host cell integrity and inhibit those pathways or processes required by the virus. We have used molecular genetics to define the specific host cell genes regulated during infectious challenge by PRRSV (Zhang et al., 1999). Approximately 120 cDNAs were cloned based on altered levels of mRNAs during in vitro infection of porcine macrophages. These ESTs provide starting points for determining the biological role a gene product plays in disease severity or outcome for PRRSV and perhaps other viruses of pigs.

Because macrophages are distributed at portals of pathogen entry, they encounter viruses early in the infection process and have effective and broad functions against viruses. These activities can be classified as "intrinsic" and "extrinsic." Intrinsic functions are those that interact with the pathogen within the cytoplasm of the infected macrophage. Macrophages possess various degrees of susceptibility (permissiveness) or resistance to virus replication, determined in part by host genetics, the virus in question, macrophage maturity, and activation status. Concerning PRRSV, activation by INF-α or IFN-γ reduced the number of macrophages that could be infected (Duan et al., 1997; Bautista and Molitor, 1999), leading to our hypothesis that protective genetic programs can be mobilized against PRRSV. Intrinsic macrophage antiviral activities may be the most critical for PRRSV owing to its restricted replication to cells of the monocyte/macrophage lineage.

Extrinsic antiviral activities of macrophages are critical to limiting the spread of pathogens by rendering surrounding cells less permissive. This activity is not expressed by resident tissue macrophages and becomes measurable only following a stimulatory event. Extrinsic interactions are due to induced macrophage secretion of factors that cause surrounding cells to resist or impede pathogen replication. For PRRSV, there is a deficit in IFN-α production (van Reeth et al., 1999; Albina et al., 1998), suggesting that part of its pathogenesis is the abrogation of a normal response by infected cells that activates anti-viral processes in surrounding cells.

The mechanisms by which macrophages are more restrictive to PRRSV are largely unknown, although cloning of PRRSV response ESTs is providing some hints. To clone ESTs reflecting changes in gene expression during PRRSV infection, we performed differential display reverse transcription PCR (DDRT-PCR) analysis using total RNAs isolated from mock- and PRRSV-infected macrophages over a 24-hour infection period. Over 8,000 DDRT-PCR products were compared, yielding approximately 120 DDRT-PCR products that were reproducibly induced or suppressed (>2-fold difference between samples). PRRSV-regulated DDRT-PCR clones have been screened by Northern blot against total RNA from mock- and PRRSV-infected porcine macrophages. From these virus-regulated ESTs, putative function for several has been determined based on full-length cloning. This includes several genes with presumed intrinsic or extrinsic activity against viruses. Thus, key changes in porcine macrophage gene expression reflective of PRRSV infection have been identified and are being used to delineate molecular mechanisms of pathogenesis and resistance.

Putative function for selected PRRSV-response ESTs

In order to determine the putative function of selected PRRSV-response genes, we obtained full-length cDNA information for several clones by combination of cDNA library screening and rapid amplification of cDNA ends by PCR. Database searches revealed orthologues in other species or conserved functional domains. We describe three PRRSV-response ESTs below and speculate on their impact on disease.

Mx1 genes

Work has shown upregulated expression of macrophage Mx1 transcripts in response to PRRSV and pseudorabies (Zhang et al., 1999). Mx1 proteins interfere with replication of some viruses in other cell types via partial inhibition of primary transcription of parental genomes (Horisberger, 1995). Nonetheless, infectious PRRSV and pseudorabies virions were produced by macrophage cultures. This may reflect species differences since Mx1 induction alone is sufficient for resistance to influenza virus in mice, but not humans. Relevant to our work is the demonstration that Mx1 alleles in mice determine resistant or susceptible phenotypes (Horisberger, 1995). Based on Mx1 activities in other species and the fact that pseudorabies virus induces Mx1 expression in porcine mac-

rophages, this gene product is likely to be involved in host cell protection against viruses in general, either following infection directly or via IFN released by neighboring cells which harbor the virus. However, Mx1 mRNA accumulation did not prevent PRRSV or PRV replication in porcine alveolar macrophages. Thus, its importance during PRRSV infection, maintenance of infected cell homeostasis, and development of CPE is unclear.

Ubiquitin proteases

Ubiquitin proteases (UBP) comprise a protein superfamily in which more than 60 UBPs have been identified in different species (Wilkinson, 1997). We cloned a ubiquitin protease that may regulate degradation of viral proteins for MHC class I presentation or may be involved budding of the virus (Patnaik et al., 2000). Identification of a PRRSV-induced UBP is the first such protein described in pigs. UBPs specifically hydrolyze ester, thiol ester, and amide bonds to the carboxyl group of G76 of ubiquitin in which ubiquitin conjugates with target proteins that will be degraded by the proteasome. Ubiquitin modification and deubiquitination by UBPs is increasingly recognized as important protein regulatory strategies that impact cell cycle regulation, cellular growth modulation, transcription activation, antigen presentation by MHC class I, and DNA repair and differentiation (Hochstrasser, 1995; Rock et al., 1994). Porcine UBP gene expression induced by PRRSV may be involved in regulating protein metabolism via a ubiquitin-conjugated pathway. This could benefit the host cell in that removing ubiquitin from host proteins prevents them from being moved to the proteasome, helping to maintain protein levels in the face of viral disruption of host translation. Conversely, the virus may induce UBP to prevent newly synthesized viral proteins from being degraded. Finally, UBP gene induction may disrupt antigen presentation by infected macrophages, thereby greatly compromising host immune responses.

RNA helicases

The biological function of RNA helicases impacts transcription, pre-mRNA splicing, ribosome biogenesis, tRNA processing, translation, nuclear mRNA export, RNA degradation, and mitochondrial RNA processing. Like proteins, RNA molecules adopt sequence-specific secondary and tertiary structures that are required for function. Alteration of these structures provides a means of regulating RNA function through local unwinding of complex RNA structures. Therefore, RNA helicases represent key elements in the regulation of different cellular processes. Outside of the conserved functional domains, the flanking amino acid sequence and length varies greatly throughout the RNA helicases, suggesting that the variable sequence and length are associated with RNA helicase specific activities, substrate specificity or intracellular compartmentalization. We identified an RNA helicase induced by virus (RHIV-1) that showed 84% amino acid similarity to the human retenoic acid-induced gene (Zhang et al., 2000b). RHIV-1 transcripts were detected at widely diverse levels in various tissues from normal pigs. As yet, substrates for porcine RHIV-1 or its apparent human homologue RIG-1 are unknown.

Interference and regulation of host cell gene expression is a common strategy employed by viruses to enhance their own replication and gene expression. In particular, interactions between viral particles and host cell RNA processing pathways are increasingly apparent, including recruitment and utilization of host cell RNA helicases. For instance, a host cell RNA helicase targeted by the hepatitis C virus core protein can influence its activity or modulate its trans-activation ability (You et al., 1999), and human RNA helicase A bind to sequences present in HIV-1 RNAs (Li et al., 1999). This suggests a mechanism by which PRRSV recruits RHIV-1 to enhance its own RNA processing, RNA translation, or genome replication. Conversely, the enhanced level of porcine RHIV-1 transcripts may impact host cell RNA processes to increase production of critical host cell proteins in an effort to sustain normal cell metabolism during biochemical disruption by viruses. We previously reported that pseudorabies virus which has a dsDNA genome also induced RHIV-1 transcripts in porcine macrophages, suggesting a common anti-viral strategy benefiting the host cell.

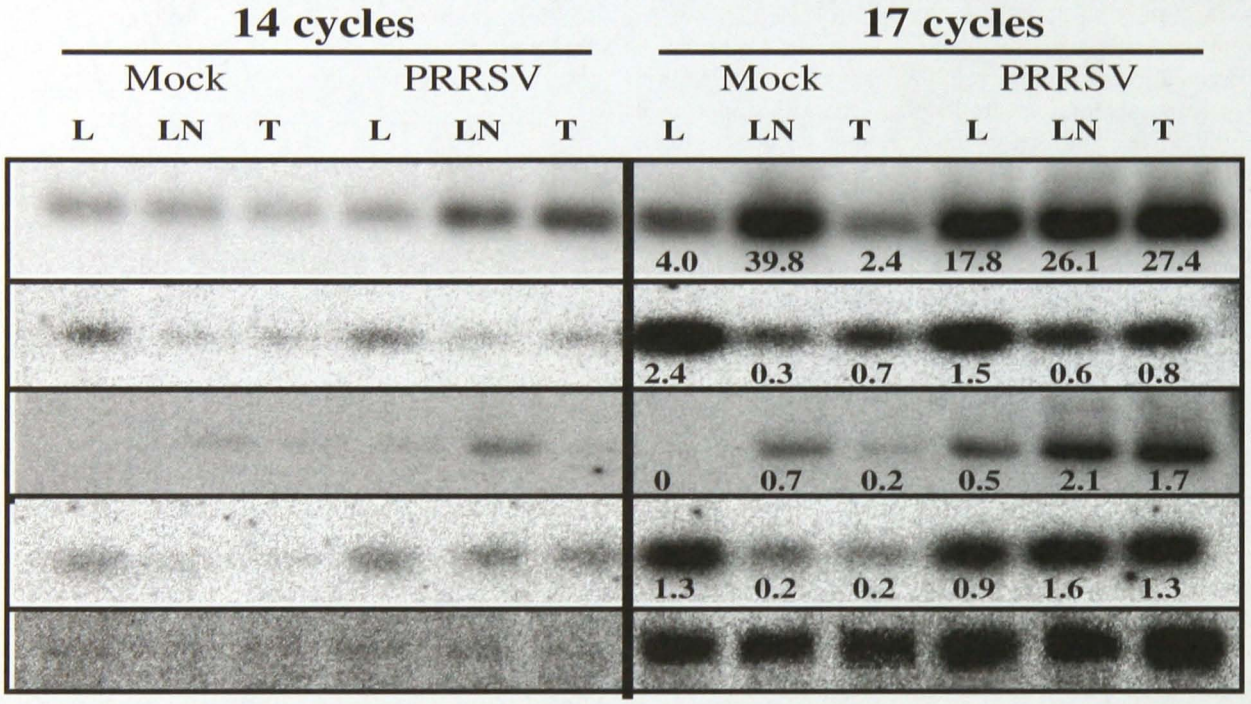
In vivo EST expression

In order to determine whether expression of ESTs identified during in vitro infection reflect events during PRRSV infection in vivo, we examined tissue-specific expression for several ESTs, including RHIV-1, UBP, and Mx1 in PRRSV-infected pigs. Tissues were collected 14 days post-infection from 2 PRRSV-infected pigs and 2 mock-infected pigs. Semi-quantitative RT-PCR demonstrated the presence of PRRSV RNA in lungs, lymph nodes, and tonsils. Amplicon levels for each tissue sample were normalized to HPRT levels, and normalized values for each tissue were compared between uninfected and PRRSV-infected animal (Fig. 1). We observed that RHIV-1 expression was increased in tonsil (T; 6.5-fold) and tracheobronchial lymph nodes (LN; 8-fold) in PRRSV-infected pigs, whereas UBP transcripts were greatly upregulated by PRRSV infection in the lungs (L; 4.5-fold) and tonsils (11.4-fold). In contrast, Mx1 transcripts were greatly induced in all tissues from PRRSV-infected pigs. These data confirm that the ESTs obtained from purified alveolar macrophages are PRRSV-response ESTs that reflect in vivo events.

Development of porcine genome maps

In parallel with the human genome initiative, significant progress has been made in the last decade with respect to

Figure 1. RT-PCR analysis of transcripts *in vivo* during PRRSV infection



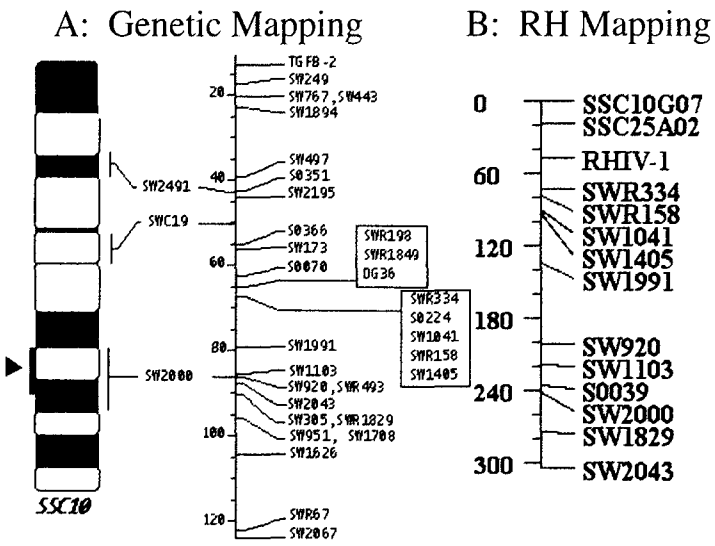
Lung (L), lymph node (LN) and tonsil (T) RNAs from PRRSV-infected pigs were reverse transcribed and amplified for 14 or 17 cycles using DDRT-PCR cDNA-specific primers for: top is UBP; second row is a novel EST; third row is Mx1; fourth row is RHIV-1; bottom row is HPRT.

development of livestock genome maps. In swine, microsatellite maps have been published (Rohrer et al., 1994), and linkage and physical maps have been constructed (Yerle et al., 1995; Rohrer et al., 1996). The recent development of a porcine radiation hybrid (RH) panel (Yerle et al., 1998) and an initial RH map spanning the entire genome (Hawken et al., 1999) permits us to fine map and order markers within a small genomic distance. Improvements in genetic maps, statistical analysis, and molecular genetics have enabled a number of traits to be genetically mapped in livestock species. In swine, associations between genetic markers and growth and carcass characteristics (Hawken et al., 1997; Wilkie et al., 1999, Paszek et al., 1999) and intramuscular fat (Janss et al., 1994) are reported. Additional resource families are being produced and phentoyped, but none address immune performance. Maps for genes controlling disease resistance/susceptibility will be enhanced by continued development of gene markers to improve map resolution. Toward this end, we have produced chromosome assignments for a number of PRRSV-response genes (see **Figure 1**; Zhang et al., 2000a and 2000b; Wang et al., 2001). To provide a fine map localization for these ESTs, we amplified across the porcine radiation hybrid panel. Co-retention of marker patterns was calculated using the RHMAP 3.0 program. Analyses provided a fine map placement for porcine RHIV-1 between SSC25A02 (29cR distant) and SWR334 (26cR distant) on SSC10 (see **Figure. 2**; and Zhang et al., 2000b). Similarly, porcine UBP, a ubiquitin protease induced by viral infection, was placed on SSC5 between SW152 (49cR distant) and S0018 (33cR distant; see **Figure 3**). In total, chromosomal localization has been achieved for 39 virus response ESTs of porcine macrophages.

Single nucleotide polymorphisms

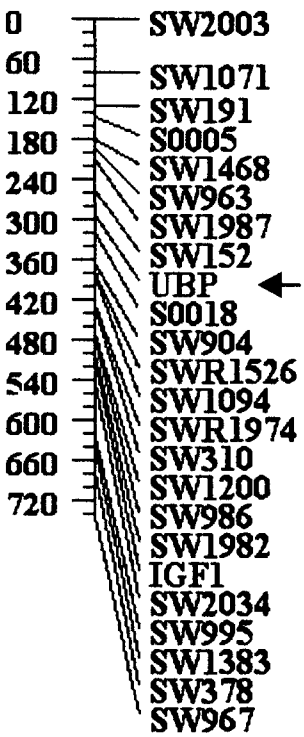
Fundamental to gene isolation is mutation identification. A range of mutation detection methods exist, each with their own strengths and weaknesses (reviewed by Cotton et al., 1998). While new high throughput SNP techniques are becoming reality, single strand conformational polymorphism (SSCP) analysis remains widely used for mutation detection because of its simplicity and versatility. In this method a segment of DNA is amplified by PCR from genomic or cDNA. This fragment is denatured and the single stranded DNA is separated by non-denaturing gel electrophoresis. Conformational changes of the single stranded mutant DNA causes a mobility shift during electrophoresis. Once a mutation is detected as the presence of bands with shifted mobility, DNA bands can be recovered from the gel matrix, re-amplified, and sequenced to identify the nucleotide change. In this way single alelles are sequenced to facilitate the unambiguous identifica-

Figure 2. Localization of porcine RHIV-1



(A) Physical location determined by somatic panel mapping; and (B) RH map of SSC10 indicating the location of RHIV-1 (Zhang et al., 2000b).

Figure 3. RH map of porcine ubiquitin protease (UBP)

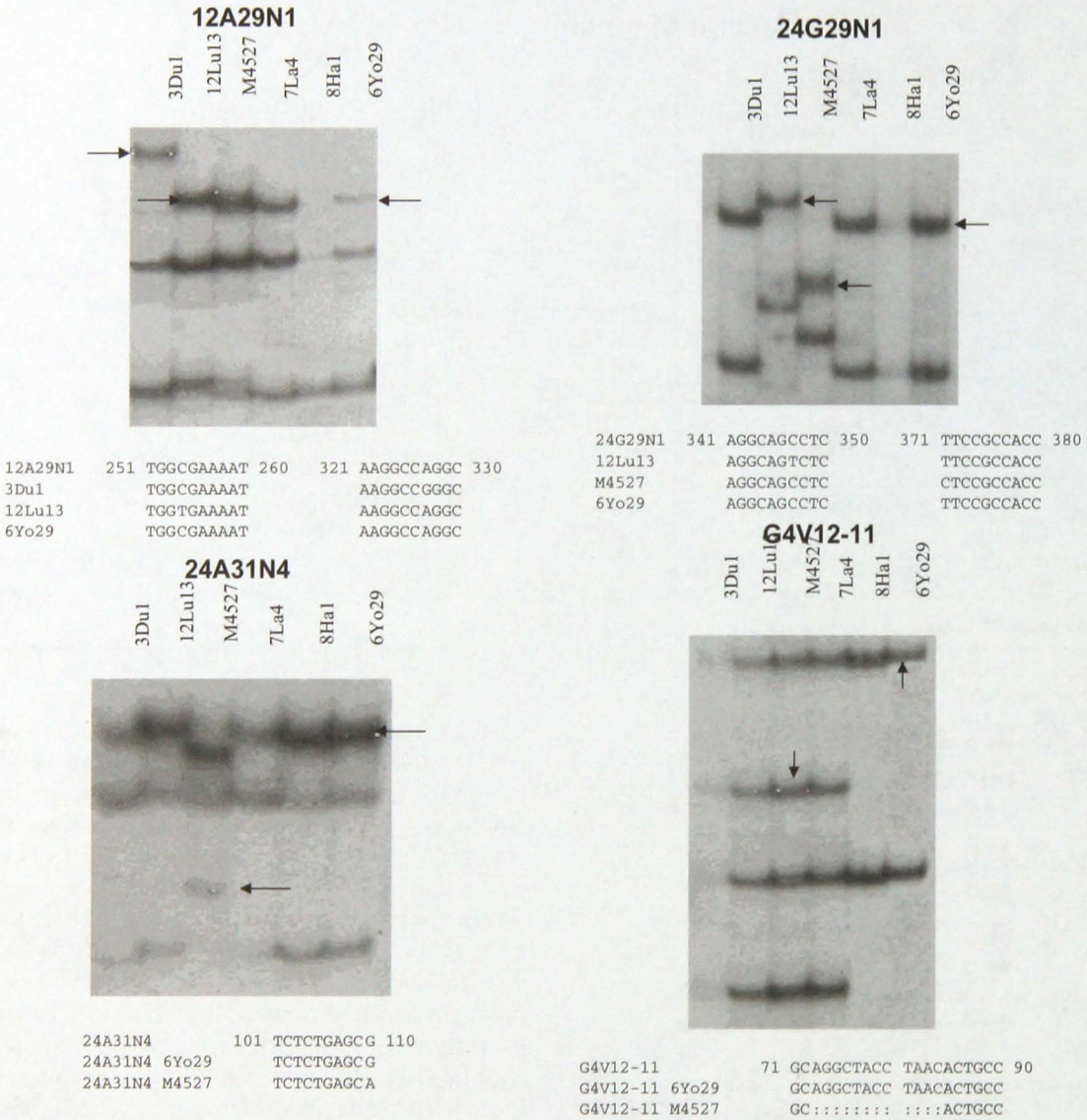


Map of SSC5 indicating the location of UBP (Zhang et al., 2000a).

tion of nucleotide change position. When codon reading frames are known, SNPs that result in changes in deduced amino acid sequence are rapidly identified, and potential changes in function can be evaluated.

Allelic variation can impact phenotype either by changing the function of a given protein. Disease resistance may result from allelic variations in specific genes which contribute to the phenotype. Evidence supports breed-specific susceptibilities to certain PRRSV pathologies (Halbur et al., 1997), suggesting that genetic differences may impact disease severity. Thus, we recently completed a first screen for polymorphisms in selected PRRSV-response ESTs (Hawken et al., 2001). SSCP analysis was used to detect allelic variants of PRRSV regulated transcripts. For this analysis, DNA samples from each of six swine breeds (Yorkshire, Meishan, Landrace, Lanyu, Duroc, and Hampshire) was amplified via the PCR using primers designed to amplify small regions of the original EST. SSCP analysis of amplified PCR products were conducted via electrophoresis on non-denaturing MDE gels (0.5XMDE, 0.6%TBE) run at a constant temperature (12°C or 22°C). Following autoradiography, allelic variants were cut from the gel and re-amplified for sequencing. SSCP analysis was conducted on 52 PRRSV-response ESTs (see **Table 1**). SNPs were detected in 26 (50%) of these ESTs via SSCP analysis at either or both 12°C and 22°C. Sequencing of allelic variants in each of the 26 ESTs revealed base pair substitutions or insertion/deletions in 19 of these 26 ESTs (examples are shown in **Figure 4**). Substitutions include 14 G/A, 8 C/T, one 12bp insertion/deletion and one 6bp insertion/deletion. These data indicate that high frequency of allelic polymorphism in PRRSV-response genes.

Figure 4. SSCP detection of PRRSV response EST polymorphisms



SSCP images and sequencing results of four PRRSV response transcripts. Arrows indicate bands that were isolated and sequenced. Sequence polymorphisms are presented below the image. The first sequence was from the initial EST cloning (Wang et al., 2000). The sequences underneath are the result of sequencing single stranded products excised from the SSCP gel. Polymorphisms are indicated. The numbers on the first sequence shows the position of the sequence in the original EST sequence. 3Du1: Duroc, 12Lu13: Lanyu, M4527: Meishan, 7La4: Landrace, 8Ha1: Hampshire, 6Yo29: Yorkshire

Conclusions

Taken together, the above data demonstrate that:

- the molecular program of PRRSV-infected macrophages is altered;
- expression genes that impact mRNA stability and protein trafficking is enhanced;
- expression of the anti-viral Mx1 gene is induced by PRRSV;
- PRRSV response genes are being placed on the porcine genome map; and,
- SNPs and deletions are apparent in a large number of PRRSV-response genes.

If we are to understand macrophage-pathogen interactions, we must begin by delineating molecular changes that occur within the infected macrophage because the complement of genes expressed will affect the outcome of immune challenge. Our results confirm that we have identified and mapped critical ESTs that reflect primary

Table 1. Porcine macrophage PRRSV-response ESTs (modified from Wang et al., 2000)

Marker name	Genbank Accession No.	Highest similarity, accession number	EST length	Regulation by PRRSV	Swine chromosome location	PCR product size	PCR anneal temp.	SSCP analysis 12°C	SSCP analysis 22°C	Best SSCP temp.	Results, position of SNP within the entire EST length
0C29N1	AW231956	Mus musculus translation represor NAT1 mRNA, U76112	263	Inhibited	2	120	55	X	X		
12A16N1	AW231894	Bovine 80-87 kd myristoylated alanine-rich C kinase substrate protein gene, M24638	297	Induced	1	149	60	X	X		
12A16N3	**AW231907**	**Interferon-stimulated protein, 15 kDa**	**558**	**Induced**	**8**	**167**	**50**	(	(	**22**	**Pos° 206 G/A, Pos° 320 C/T**
12A29N1	**AW231955**	**None**	**519**	**Inhibited**	**7**	**172**	**60**	(	(	**12**	**Pos° 254 C/T, Pos° 327 G/A**
12A29N11	AW231954	Sus scrofa alveolar macrophage-derived chemotactic factor-II mRNA, M99368	187	Inhibited	8	154	50	X	X		
24A19N1	AW231908	Homo sapiens Lsm 3 protein, NM-014463	415			170	55	?	X		
24A28N3	**AW231952**	**None**	**262**	**Induced***	**17**	**112**	**50**	?	(	**22**	**Pos° 28 of PCR product G/A**
24A29N8	AW231964	Homo sapiens mRNA; AL050195	365	Induced	1	166	58	(	?	12	No SNP detected, need to re-sequence ends
24A31N2	AW231899	None	224	Induced	1	100	60	X	X		
24A31N4	**AW231902**	**None**	**179**	**Induced**	**2**	**147**	**60**	(	(	**22**	**Pos° 110 G/A**
24C27N1	**AW231950**	**Homo sapiens dual specificity phosphatase 1 mRNA, NM_004417**	**396**	**Induced***	**16**	**211**	**58**	(	(	**12**	**Pos° 287 G/A**
24C27N4	AW231951	Homo sapiens clone, AF055003	222	Induced	6	114	50	X	X		
24C32N2	AW231905	Sus scrofa mRNA for 2'-5' oligoadenylate synthetase p42 isoform, AJ225090	259	Inhibited*	14	166	55	?	?		
24G17N2	AW231910	None	443	Induced	14	269	58	X	(	22	No SNP detected, need to re-sequence ends
24G18N1	AW231896	Homo sapiens cDNA clone, AI937234	168	Inhibited	1	103	60	X	X		
24G19N1	AW231937	None	137		9	120	60	X	X		
24G24N2	**AW231942**	**Bos taurus D4-GDP-dissociation inhibitor mRNA, AF182001**	**175**	**Inhibited**	**5**	**138**	**55**	**?**	(	**22**	**Pos° 78 C/T**
24G29N1	**AW231957**	**GTP-binding protein MX2, AF239824**	**498**	**Induced***	**13**	**204**	**55**	(	(	**12**	**Pos° 347 C/T, Pos° 371 C/T**
24G29N2	AW231958	None	152	Induced*	11	153	60	X	X		
24G31N1	**AW231898**	**Melanoma differentiation associated protein-5 Homo sapiens**	**489**	**Induced***	**15**	**250**	**50**	(	(	**12**	**Pos° 323 G/A**
2C18N2	AW231943	None	168	Induced	2	104	60	X	X		
2C19N1	**AW231944**	**None**	**785**	**Induced**	**3**	**250**	**60**	(	(	**12**	**Pos° 454 G/A**
2C19N2	**AW231945**	**None**	**434**	**Induced**	**14**	**179**	**60**	(	(	**22**	**Pos° 43, 44, or 45 - 6 bp insertion**
2C20N1	AW231946	Homo sapiens cDNA: FLJ22698 fis, AK026351	303			175	55	X	X		
2C22N1	AW231947	Sus scrofa porcine endogenous retrovirus PERV-MSL mRNA, AF038600	481	Induced	13	195	58	(	(	12	No SNP detected, need to re-sequence ends
2C22N3	AW231949	Novel human gene on chromosome 1, AL096858	301	Induced	6	173	58	(	X	12	No SNP detected, need to re-sequence ends
2G19N1	AW231935	None	188	Induced	13	103	55	X	X		
2G19N2	AW231936	None	189	Induced	12	138	58	X	X		
A10M24-11	**AW231911**	**Sus scrofa cytochrome C oxidase polypeptide, F14834**	**167**	**Inhibited**	**9**	**114**	**58**	(	(	**12**	**Pos° 64 G/A/T, Pos° 91 G/A, Pos° 109 T/C**
A12V24-11	AW231913	None	292	Induced	8	277	55	(	(	12	No SNP detected, need to re-sequence ends
A2V16-22	**AW231914**	**Homo sapiens BAC sequence AL121655**	**234**	**Induced**	**3**	**122**	**55**	(	(	**12**	**Pos° 164 G/A**
A4M24-12	AW231915	None	377	Inhibited	9	199	60	X	X		
A4M24-43	**AW231917**	**None**	**306**	**Inhibited**	**14**	**199**	**60**	(	(	**12**	**Pos° 187 C/T**
A4V12-22	AW231918	Bovine αS1-casein (84%)	187	Induced		129	55	X	X		
A8M24-44	**AW231919**	**Rat 60S ribosomal protein L22**	**485**	**Inhibited**	**9**	**158**	**50**	(	(	**12**	**Pos° 401 G/A**
A9M24-23	AW231968	Sus scrofa mRNA for annexin I, X95108	239	Inhibited	1	164	55	X	X		
C12V16-22	**AW231921**	**Homo sapiens 12p13.3, chromosome 12 open reading frame, unnamed protein**	**329**	**Induced**	**5**	**253**	**55**	(	(	**12**	**Pos° 227 G/A**
C12V24-11	AW231922	None	295	Induced	8	250	55	X	X		
C3V16-11	**AW231923**	**None**	**166**	**Induced**	**12**	**221**	**58**	(	(	**12**	**Pos° 89 G/A, Pos° 108 G/A**
C7V16-11	**AW231925**	**None**	**217**	**Induced**	**6**	**106**	**55**	(	(	**22**	**Pos° 119 G/A**
C7V16-12	AW231926	Bovine GalT mRNA for beta-1,4-galactosyltransferase, X14558	217	Induced	10	155	50	X	X		
C7V16-31	AW231927	Bos taurus thioredoxin mRNA, AF104105	238	Induced	1	112	50	X	X		
C7V16-41	AW231928	Bos taurus CI-B12 mRNA for ubiquinone oxidoreductase complex, X63213	320	Induced	15	124	58	X	X		
C7V16-52	AW231929	Homo sapiens mRNA for galectin-3, AB006780	415	Induced	1	212	50	X	X		
G2V12-12	**AF102505**	**None**	**187**	**Induced**	**9**	**112**	**60**	**?**	(	**22**	**Pos° 148 C/T**
G4V12-11	**AW231931**	**None**	**268**	**Induced**	**14**	**157**	**60**	(	(	**12**	**Pos° 72, 12 bp deletion**
G9M24-32	AW231932	Human DNA sequence on chromosome 20, AL049540	156	Induced	17	119	58	X	X		
G9M24-33	AW231933	None	156	Inhibited	10	105	50	X	X		
G9M24-44	AW231934	Homo sapiens cDNA clone, AW027135	191	Induced	8	112	55	X	X		
G12V24-12	AF102506	Porcine *Mx* 1	317	Induced	13	200	50	(	(	22	Not sequenced
A5V12-11	AF102503	Porcine RHIV-1	214	Induced	10	189	55	X	X		
C3V16-31	AW231924	Sus scrofa adipocyte cDNA clone, AU055644	235	Induced	4	150	52	X	(	22	No SNP detected, need to re-sequence ends

ESTs indicating a polymorphism via SSCP analysis and sequencing are presented. X: no SSCP allelic variant detected, (: allelic variant detected, ?: unknown.

changes in macrophage gene expression due to PRRSV infection. This information permits candidate gene analysis for understanding PRRSV pathogenesis in the pig. Defining the critical molecular components that determine the balance between viral clearance and replication may have great importance in the design of marker-assisted selection programs and the development of chemotherapeutic interventions.

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