Mapping Bovine Genes for Viral and β-interferon Sensitivity in Somatic Cell Hybrids

by

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Submitted in Partial Fulfillment of the Requirements of the University Undergraduate Fellows Program

1982-1983

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April 1983

Abstract

The cattle genome contains a gene for vesicular stomatitis virus (VSV) sensitivity, possibly coding for a VSV receptor molecule. This was demonstrated by a VSV challenge to cattle-hamster hybrid clones segregating cattle chromosomes. Linkage of this gene to cattle genes coding for different enzymes was not established with the small mumber of clones examined in this study.

The gene for a β-interferon receptor (IfRec) was found to be linked to the superoxide dismutase-1 (SOD-1) locus, with only one discrepancy in the nine clones examined. Since this linkage has been reported in humans on chromosome 21 and mice on chromosome 16, its conservation in cattle is interesting from both evolutionary and medical perspectives.

I would like to thank Kathy Fuxa and Yvonne Moll for the help and assistance they gave me while working on this project.

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Introduction [a]

In the recent years of genetic engineering and manipulation, relatively little research has been done with the cattle genome. Much of the problem is because cattle have 60 small chromosomes and produce relatively few offspring after long gestation periods. This has discouraged conventional methods of gene mapping with established gene markers. Yet gene mapping could have untold benefit on the beef and dairy industry to improve production and selection of superior individuals.

Ephrussi and Weiss (1965) developed a cell fusion technique call somatic cell hybridization. This revolutionized gene mapping studies of humans and has potential in a variety of other mammals. The process fuses two cells from different species to provide a hybrid population. When cattle cells are fused to a transformed cell line, hamster or mouse usually, cattle chromosomes will be lost at random or segregated (Weiss and Green, 1967). However, the full genome of the transformed cell line is kept. Figure 1 demonstrates the retention of known cattle genes as a percentage of the total genes retained versus the number of clones. It illustrates the other problem of cattle gene mapping studies: not enough gene markers. The hybrid cells, after fusion, are separated and cloned, and later analyzed for gene markers (Nabholz et al., 1969, Ruddle et al., 1970, Santachiara et al., 1970).

Up to this date gene markers have been primarily isoenzymes, as given by Figure 2, determined by electrophoresis. Other methods include DNA probes, for example, but electrophoresis

a. The format followed is that of *Cell*.

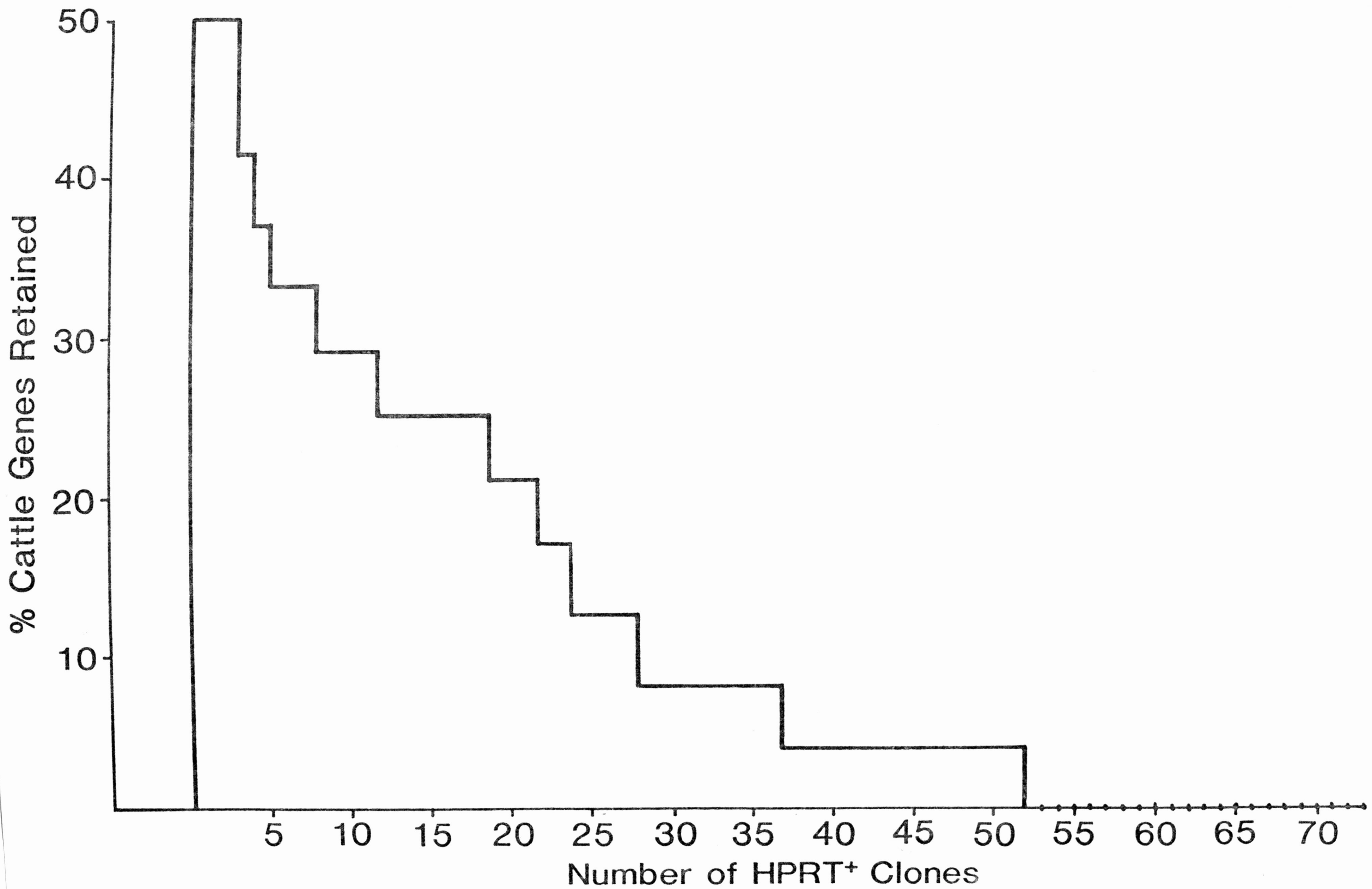

Figure 1. Graph of the percent of known cattle genes (total number 22) versus the number of hybrid clones examined for them.

Syntenic Group	Genes	Syntenic Group	Genes
1 (x)	G6PD HPRT PGK GLA	10	IDH1
2	PGD ENO1	11	PEPC
3	LDHB TPI PEPB	12	PGM2
4	ME1 PGM3 SOD2	13	GPI
5	MPI	14	AK1
6	MDH2	15	ACY1
7	LDHA	16	PKM2
8	PGM1	17	GUS
9	SOD1	18	GSR

Figure 2. Cattle gene map as it stands now. Of the syntenic groups, only 1 is assigned to a chromosome (X), and 12 chromosome homologues have no markers at all.

is used more widely due to its ease. However, Dr. Joseph Cummins at T.A.E.S., Amarillo (unpublished data) noted in 1982 that hamster cell lines were not as susceptible to vesicular stomatitis virus (VSV) as were cattle cells and some of the cattle-hamster hybrid clones. Those hybrid cells which did not appear as susceptible to the virus challenge responded more similarly to the hamster cells. The same appeared true for the sensitivity of hybrid cells to cattle β-interferon (Cummins, unpublished).

Using a panel of hybrid clones segregating cattle chromosomes I looked for linkages of isoenzymes fo the two potentially important genes discovered by Cummins.

Methods and Materials

The two progenitor cell types used were cattle leukocytes and a transformed Chinese hamster cell line. The cattle leukocytes were taken from random cattle and the blood centrifuged to retrieve the leukocytes. The hamster cells were from a HPRT deficient transformed E36 line. The HPRT deficiency was useful for selecting hybrid clones. Hybridization was accomplished by "pancake fusion" in polyethylene glycol as developed by O'Malley and Davidson (1977). The hybrid clones were selected for in a medium containing hypoxanthine (HAT). This killed the HPRT deficient hamster cells and any hybrids that did not retain a functional cattle HPRT gene. Since cattle leukocytes will not grow in culture, only the HPRT positive hybrids will survive. These hybrids are removed and cloned to produce sufficient quantities for analysis.

The hybrids used had been frozen previously. They were rethawed and grown up in HAT medium. Once in sufficient quantities, the clones were sent to Amarillo to be assayed for VSV sensitivity in the laboratory of Dr. Joseph Cummins. The clones were challenged with VSV and then sensitivity was measured by dye uptake in live cells by a modification of Armstrong's dye-binding test (Armstrong, 1971). Sensitivity was determined by plaque size. Increased plaque size was a positive indication of sensitivity to VSV.

At the same time, cells previously determined to be VSV sensitive were exposed to cattle β-interferon and then challenged with VSV. This was again examined by the dye-binding test. Those cells that escaped lysis and took up the dye were determined to have been protected by the interferon. Since these clonces are responsive to exogenous interferon, they were considered positive for the IfRec gene. Sensitivity to VSV was again determined by plaque size.

Electrophoresis was used to establish whether the IfRec gene might be linked to any of the isoenzymes identified in Dr. James Womack's laboratory at Texas A&M. These enzymes are those listed in Figure 2. Concordency or gene syteny, such as the example in Figure 3, of IfRec with another enzyme was sought by use of electrophoresis on a cellulose acetate plate (Womack, personal communication).

Results

Preliminary studies on VSV sensitivity were inconclusive for gene linkage with known isoenzymes. However, there was

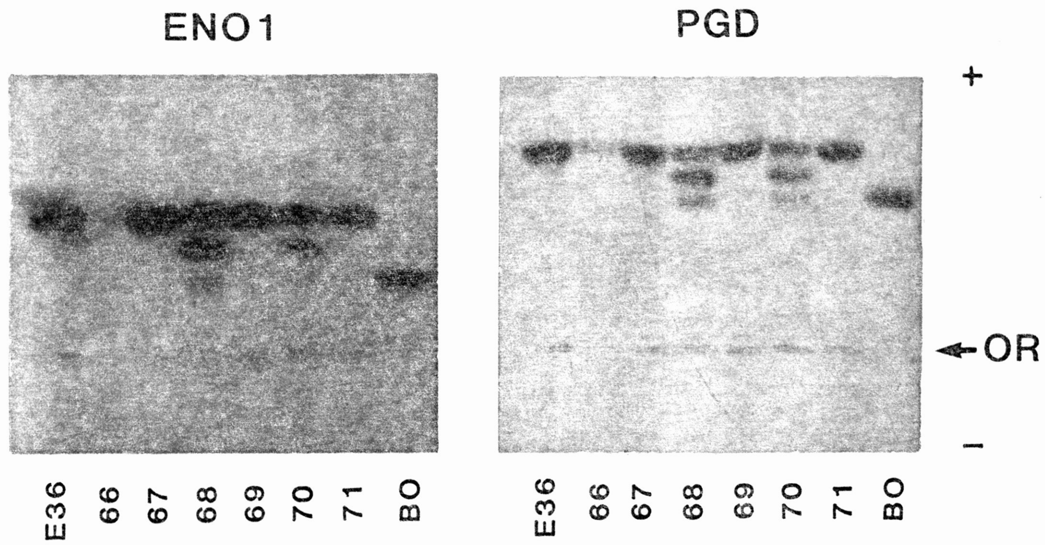

Figure 3. An example of concordancy. The two enzymes, ENO-1 and PGD are always found together. The two hybrid clones positive for the cattle enzymes are 68 and 70.

definite evidence for a VSV receptor gene in cattle. Table 1 shows the differences in plaque size between those hybrid clones determined to be positive or negative for VSV sensitivity. Studies to confirm whether the VSV receptor gene is linked to another isoenzyme or it is a new gene marker were not available. Table 2 shows the relation of the VSV receptor gene to the other isoenzymes.

Of the isoenzymes examined, only SOD-1 appeared to be linked to the IfRec. One discrepency (Bo12) was noted in the nine hybrid clones tested. Table 3 shows the gene-clone matrix of nine hybrid clones and the isoenzymes tested. A picture of a SOD-1 gel was not included due to the lack of contrast in this particular isoenzyme staining procedure.

Discussion

The discovery of receptors for specific viruses in cattle is significant. It conjectures possibilities of cattle, due to mutated VSV receptor genes, that are more resistant or susceptible to the disease. Identification of such cattle for future breeding programs could be useful in developing a line of VSV resistant cattle. If cattle have a receptor gene for VSV, it is quite possible other virus receptor genes exist. At present this would be useful mostly as gene markers for gene mapping. In the future, conceivably, this could be used as a procedure for selecting disease resistant cattle.

The linkage data collected for VSV sensitivity was inconclusive. It is possible the VSV receptor gene may be linked with ENO-1 and PGD (both linked genes) or LDHA, or it may be

Table 1. VSV Sensitivity in tested hybrid clones. E36 is the hamster line and BT is the cattle leukocytes. These were used as the controls.

CLONE	PLAQUE SIZE (mm)		CATTLE PHENOTYPE
	Challenge 1	Challenge 2	
E36	5.3	4.2	−
BT	8.5	8.5	+
Bo1.1	3.5	3.3	−
Bo6	8.5	10.3	+
Bo7	5.8	4.6	−
Bo8.1	8.4	8.6	+
Bo10	4.5	3.8	−
Bo16	10.1	7.8	+

Table 2. Gene-clone matrix of VSV receptor gene. A "0" designates not determined from the gel run. Note that the hybrid clones here are not necessarily synonymous with those in Table 3.

	HYBRID CLONES					
ENZYMES	Bo1.1	Bo6	Bo7	Bo8.1	Bo10	Bo16
VSV	–	+	–	+	–	+
ACY-1	–	–	–	–	–	–
ENO-1	–	+	–	+	+	–
GPI	–	+	–	–	–	–
GSR	–	–	–	+	–	–
GUS	–	–	–	–	+	+
IDH-1	–	–	–	O	–	–
LDH-A	–	+	–	+	–	–
LDH-B	–	–	–	+	+	–
MDH-2	–	+	–	–	+	–
ME-1	–	O	–	+	–	–
MPI	–	–	–	–	+	–
PEP-C	–	O	–	–	–	–
PGD	+	+	–	+	+	–
PGM-1	–	+	–	–	+	–
PGM-2	O	O	O	O	O	O
PKM-1	–	–	–	–	–	–
SOD-1	+	+	–	O	O	–
TPI	–	–	–	+	O	O

Table 3. Gene-clone matrix of IfRec. A "0" designates not determined from the gel run. Note that the hybrid clones here do not necessarily correspond to those in Table 2.

ENZYME	Bo8.1	Bo8.2	Bo9	Bo10	Bo12	Bo42	Bo47.1	Bo50.1	Bo70.1
IfRec	−	−	+	−	−	+	+	−	−
SOD-1	−	−	+	−	+	+	+	−	−
ACY-1	+	+	−	−	−	−	+	+	+
ENO-1	+	+	+	+	+	+	+	+	+
GPI	−	−	+	−	−	+	−	+	−
GSR	+	−	+	−	−	+	+	+	−
GUS	−	−	−	+	+	+	−	−	0
IDH-1	+	+	−	−	+	−	+	+	−
LDH-A	+	+	+	−	−	−	−	−	−
LDH-B	+	+	+	+	+	+	+	−	−
MDH-2	−	−	+	+	−	+	+	−	+
ME-1	+	+	+	−	+	−	+	−	+
MPI	−	+	+	+	+	+	+	−	−
PEP-C	−	−	+	−	+	−	+	−	+
PGD	+	+	+	+	+	+	+	+	+
PGM-1	−	−	−	+	−	−	−	−	−
PGM-2	−	−	−	−	+	−	−	−	−
PKM-1	−	−	−	−	−	0	−	−	+
TPI	+	+	+	0	0	+	+	−	−

an independent gene on another chromosome, and therefore a new gene marker. This would be useful since there are twelve cattle chromosomes that have no markers as of now.

The IfRec and SOD-1 gene linkage is very exciting since it is also observed in humans on chromosome 21 and in mice on chromosome 16 (Cox et al., 1980, Lin et al., 1980). There was only one discrepancy which could have been due to a broken chromosome, fusion of a piece of chromatin on another chromosome or possible damage to the IfRec gene so it was not expressed. This is still fairly conclusive evidence for the IfRec-SOD-1 gene linkage in cattle.

In humans the SOD-1 and IfRec linkage is considered to be on the same arm of chromosome 21 involved in trisomy 21 or Down's Syndrome (Cox et al., 1980). A question raised is whether SOD-1 or IfRec are involved in this disease. Furthermore, would trisomey of the chromosome carrying these genes in mice and cattle produce effects similar to trisomy 21? If so, research with these animals could lead to more valuable information in understanding Down's Syndrome.

The other interesting aspect of the SOD-1 and IfRec linkage is that the three species are very disimilar. Rodentia, artiodactyla and primates represent divergent branches of the mammalian tree. That a gene linkage is highly conserved in the three species is interesting from an evolutionary standpoint. Linkage of autosomal genes in such divergent mammals implies a possible selective advantage for this chromosomal block. This requires more work, such as assigning IfRec and SOD-1 in cattle to a

chromosome. Another task is to look for the linkage in other mammals and/or vertebrates.

Conclusion

A gene for VSV sensitivity in the cattle genome has been conclusively demonstrated. This gene is suspected to code for a receptor molecule. The gene was not conclusively linked with any other cattle gene markers (isoenzymes) in this study, so it may be on a separate chromosome from the isoenzymes established so far.

SOD-1 and IfRec appear to be linked in cattle. This has medical implications because of the same linkage on chromosome 21 in humans which is involved in trisomy 21. It has evolutionary significance becasue the linkage is conserved in mice, humans and cattle.

Karyotyping was not possible within the scope of this project, so the IfRec—SOD-1 syntenic group was not assigned to a chromosome. When in the future karyotyping is done, the cattle homologue of human chromosome 21 and mouse chromosome 16 will be identified.

Literature Cited

Armstrong, J.A. 1971. Chromosoma 17:1-10

Cox, D.R., Epstein, L.B. and Epstein, C.J. 1980. Proc. Natl. Acad. Sci. USA 77:2168-2172.

Euphrussi, B. and Weiss, M.C. 1965. Proc. Natl. Acad. Sci. USA 53:1040-1042.

Lin, P., Slate, D.R., Lawyer, F.C. and Ruddle, F.H. 1980. Science 209:285-287.

Nabholz, M., Miggiano, V. and Bodmer, W. 1969. Nature (London) 223:358-363.

O'Malley, K.A. and Davidson, R.L. 1977. Somat. Cell Genet. 3:441-448.

Ruddle, F.H., Chapman, V.M., Chen, T.R. and Klebe, R.J. 1970. Nature (London) 227:251-257.

Santachiara, A.S., Nabholz, M., Miggiano, V., Darlington, A.J. and Bodmer, W. 1970. Nature (London) 227:248-251.

Weiss, M.C. and Green, H. 1967. Proc. Natl. Acad. Sci. USA 58:1104-1111.

"Is there any more?" said Pooh

"No." said Rabbit.

"I thought not." said Pooh.

--probably misquoted from

Winnie-the-Pooh by A.A. Milne