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Dear Inder:

Many many thanks for sending the cDNA so promptly---it arrived only 3 days after I spoke with you. Though all the exposures of the three filters I annealed with it are not yet out, I have come to some tentative conclusions that I will share with you.

38 revertants were isolated from the ASV transformed rat cells infected with Natalie Teich's stock of MuLV (said to be Moloney, obtained from Jan Hartley and apparently cloned fairly recently). Thus far I've screened about 33 with restriction digests of the single provirus (ASV). Four have lost the entire ASV provirus and all but seven of the rest have a perfectly normal provirus and are probably nonconditional src mutants. This accords well with results with revertants obtained from the same line without MLV infection---a couple of those had lost the provirus by an unknown mechanism (prob. chromosome loss I would guess) and the rest had src lesions (we could rescue td viruses, the RNA was normal, p60 generally present but kinase activity reduced or absent).

The seven interesting ones fall into three groups. The first (7) was represented by a single revertant which has an insertion of ca. 5.5×10^6 Mr near the upstream end of the ASV provirus. The insert appears to anneal with MLV cDNA, lacks RI or Xho sites, has Bam and Bgl II sites in positions which do not accord with the MoMLV clone 1 map. In addtion, the left end of the ASV provirus (upstream end) is deleted (ca. 10^6 daltons gone). There seem to be about 2 or 3 MLV proviruses in this clone. The second type was isolated three times, all presumably the same cell in view of the restriction data. This also has an insertion of about 5×10^8 mr, but not quite at the upstream end of the ASV provirus (prob about 500-1000 bases from the end). The insert has sites for Bam, RI, and Bgl II which don't bear any obvious relation to the Mo-MLV map, but the new fragments detected with ASV appear to be detected with MLV cDNA. The only difficulty is that there are a lot of new MLV proviruses (?around 10) and the superimposition of bands could be a coincidence. So far, there is no evidence for a deletion in the ASV at the insertion site, but more mapping is in progress---a couple of hundred bases could easily be gone without my having detected the deletion thus far. The three subclones of this type have very similar if not identical patterns of fragments seen with ASV and MLV probes; hence my belief that they are identical species isolated three times (they are from the same infection and from the same plate after BUdR selection against transformed cells). The third type is represented by three clones. There is unequivocal evidence for a deletion of at least 0.4×10^8 Mr from the

left end of the ASV provirus, but thus far all the data can be accounted for without invoking an insertion. Interestingly, the three have the same ASV pattern (with three enzymes) but very different (totally different save for endogenous bands) MLV patterns, suggesting that the event initiating reversion antedated the infection I think an unusual deletion of about 1.1×10^6 Mr (mostly viral DNA, ?some linked cell DNA) occurred in these cells prior to infection, and that I then isolated the revertant three times, each clone bearing MLV proviruses at different (irrelevant)sites. But more data is pending.

Needless to say, we will need to clone these oddballs to understand them properly. In addition, Nancy Quintrell is currently looking at the ASV RNA by Northern transfer. Doubtless I will soon ask for various MLV DNA's to look at the MLV RNA's. If the 3'5' structure is a promoter at either end of the provirus, we would expect to find RNA's with the 5' sequence of MLV and linked ASV sequences, though it is possible such transcripts are destroyed rapidly if unsuitable for processing (splicing). We have seen candidate RNA's of this sort in proviruses inserted into cellular DNA, since they anneal with cDNA5' but not with cDNArep. I am about to do two additional things: look for reversion of the second type to the transformed state as a consequence of excision; and to try to retransform type I or type III by superinfection with MLV , hoping to place a 3'5' unit at a proper position to allow expression of ASV src.

We'll talk more about this and how you want to work whatever might be done collaboratively when I return in the first or second week in July.

+ of MLV DNA

Cheers,

Howard