

Stehelin, D., Varmus, H.E., Bishop, J.M., and Vogt, P.K. (1976) DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260, 170-173.

Background and significance: Decades earlier, variants of certain bird (avian) viruses had been identified that could induce tumors when they infected otherwise normal birds. Avian sarcoma virus (ASV) was one such virus that had been studied extensively. Note that the parent virus from which ASV derived does NOT cause tumors. ASV was already known to be what we now call a "retrovirus", with an RNA genome and reverse transcriptase for converting the RNA to DNA upon infection. Starting with the tumor-causing ASV, "td" (transformation defective) variants were isolated that could no longer induce tumors and these were thought to carry mutations in the gene that caused the sarcoma. Some such variants deleted the sarcoma-causing gene and this paper studies the RNA (converted to DNA for their experiments) that is present in sarcoma-causing ASV but absent in td deletion mutants. The paper correctly concludes that the tumor-causing gene is not really a viral gene. Instead, it is a slight variant of a normal avian gene, now called the *src* oncogene (for sarcoma). Their evidence for this seems indirect to us today, but in essence it amounts to showing that the *src* oncogene sequence is present in a wide variety of birds, including ones that are not infected with ASV. Because of the way retroviruses reproduce and transmit themselves, on rare occasions such oncogenes can be acquired and carried by the viral particles, making it infectious. This was the first identification of a gene that directly causes cancer, a mechanism now thought in part to underlie nearly all cancer. The normal version of the *src* gene is now known to encode a protein kinase that mediates response to growth factors during normal development. The oncogenic *src* variant stimulates cell proliferation in the absence of the normal growth factors. Bishop and Varmus won the Nobel Prize in 1989 for this work.

Explanation of some terms:

sarcoma - a tumor of connective tissue or muscle

neoplastic transformation - conversion to a cancer cell

cistron - gene

C_0t (X-axis, figure 1) - proportional to the time required to hybridize to form duplex DNA, which in turn is related to the concentration of the hybridizing species in the reaction.

hydroxyapatite - binds double-stranded DNA but not single-stranded DNA.

transduction - when a virus packages cellular DNA into the viral particle, rather than the appropriate viral DNA and then transfers it into the recipient cell.

Suggestions for reading the paper:

1. Read the center paragraph of the right column on page 172 first, starting "We have shown previously..."
2. Don't get too sidetracked by the fact that the "td" variants also hybridize to chicken DNA (though you should try to answer that question).

3. Don't get bogged down in C_{ot} methods - they aren't used any more. Think of them more or less as a laborious and less informative version of Southern blots and accept the authors' conclusions.
4. If you get really bogged down by one point, move on and try to go back later when you have the whole paper completed.

Questions to answer:

1. How do you suppose it was determined that the "td" variants of ASV had deleted part or all of the oncogene (as these genes are now called)?
2. What is the significance of the evolutionary conservation of the oncogene?
3. Why do you suppose "td" variants also hybridized to the chicken genome but not others? (Hint - read in a textbook about how retroviruses replicate and think about the biology of viral/host interactions)

PS This is a technically very difficult paper, especially to early in the course, but it is perhaps the most pivotal paper in the history of cancer genetics so we cover it anyway.

Paper recap.

This paper reported the first molecular isolation of a gene that causes cancer, an oncogene. The key finding in the paper is that this virally transmitted oncogene is the same as a normal gene whose DNA sequence is conserved throughout birds and to a lesser extent mammals as well. Radioactively labeled oncogene was purified away from the viral RNA that carries it by: 1) using reverse transcriptase to copy the oncogene-bearing viral RNA into cDNA, and 2) hybridization to the RNA from a viral variant that had lost the oncogene and removal of the double stranded nucleic acid. The pure labeled oncogene (and a labeled viral DNA in parallel) was used as tracer in a series of solution hybridization experiments, mostly with various bird genomic DNAs as driver (the same experiment would be done today using a Southern blot, which is essentially the same experiment but with the driver size fractionated and immobilized on a membrane - think about it!). These experiments showed that the chicken genome has a nearly exact copy of the viral oncogene and that this gene is conserved among birds. In an experiment we will discuss only briefly, they used heat denaturation to measure how exactly matched the labeled oncogene/genomic DNA duplex was.

These results suggested that the oncogene has a normal function in these animals, one that is evolutionarily conserved and that is presumably not to cause cancer (!). Within a few years, these and other scientists had sequenced the *src* oncogene, had shown that the virus merely causes overexpression of the *src* gene, and that *src* encodes a protein kinase implicated in regulation of cell proliferation. Bishop and Varmus, the two principal investigators on the paper we read, won the Nobel Prize jointly in 1989 for this work. It is now known that the *src* kinase helps mediate response to a number of growth factors that control cell proliferation.