Gene Scanning with Block Mutations

A new method allows researchers to insert mutations at specific sites and determine which DNA segments are needed for a gene's activity

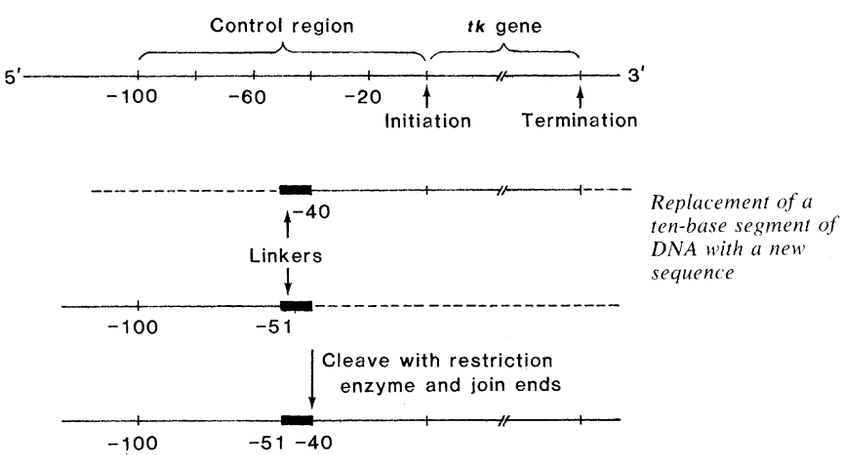
A new technique has been developed for studying gene function by introducing clusters of point mutations into DNA at specific locations without causing the addition or deletion of base pairs. The method, described in last week's *Science** by Steven McKnight and Robert Kingsbury of the Fred Hutchinson Cancer Research Center in Seattle, can produce mutant forms of any gene that can be isolated and cloned. By constructing a series of mutants in which the position of the block mutation is systematically moved, researchers can rapidly scan a length of DNA to see which regions are critical for the activities of the protein product of a gene, or, as in this case, which regions are involved in regulating gene expression.

Within the past 2 to 3 years, researchers have learned that the DNA sequences that regulate the first step of protein synthesis, the transcription of a gene into messenger RNA, are relatively complex in higher organisms. The control sites often extend from one hundred to a few hundred bases upstream from the transcription initiation site (*Science*, 8 May 1981, p. 653). For example, the McKnight group found that the transcription control sequences of the herpesvirus *tk* gene, which codes for the enzyme thymidine kinase, extends some 100 base pairs to the left of the first base encoding *tk* messenger RNA.

The control sequences were initially identified by systematically deleting segments of DNA from the regions around the *tk* gene to see how transcription would be altered. "But deletions do two things at once," McKnight explains. "They change the spacing between DNA segments as well as the sequence." This makes it difficult to interpret experimental results unequivocally. In addition, pinpointing exactly which bases within a long region are functionally important is difficult by the deletion method.

"What is the next step after identifying the control region?" McKnight asked. "One answer is to make point mutations instead of deletions. But it is far too much work to make single mutations for each of the 100 bases. We wanted to make clusters of point mutations."

They began by making two series of deletion mutants. In one, varying numbers of bases were removed, beginning at the 5′ end of the control region. The other had the deletions beginning at the 3′ end. They then attached to each of the newly exposed ends a linker sequence of ten bases that contains a recognition site for a restriction enzyme (the one designated Bam HI). Each of these molecules was then cloned in a plasmid vector. The cloned deletion mutants were cut out of the vector by digestion with Bam HI. Ultimately, an appropriate 5′ deletion mutant was joined to an appropriate 3′ deletion mutant to give a final product in which a ten-base segment of the original control sequence was replaced with the ten-base linker sequence.



The diagram shows an example in which base pairs −41 through −50 are replaced with linker DNA. [The bases are numbered counting upstream (or backward) from the transcription initiation site.] This requires a 5′ deletion mutant in which the bases have been removed from the left down to base −40, which then has a linker sequence added. For the 3′ deletion mutant, the bases are removed from the right through base −50 and the linker sequence is attached to base −51. After cutting the cloned molecules with the restriction enzyme, the ends are joined to make a complete control region, which has the new 10-base substitution and is attached to the gene itself. Although it might not seem so from the description, McKnight says, "It is a simple concept. In essence it uses only existing technology."

In any event, McKnight and Kingsbury constructed a series of mutants in which they began at the left end of the gene and systematically replaced ten-base segments of the control region with linker until they reached the control region's far upstream end (base −119). By testing each of these mutants in an in vivo assay for transcription, they identified three separate segments within the 100-base control region that are important for initiation.

The first of these extends from base −16 to −32. Mutations here cause a marked reduction in transcription efficiency and some also cause transcription to begin at the wrong site. This region contains the "TATA box," which had already been implicated as an important signal regulating gene transcription. Earlier results about the role of the TATA box in transcription were confusing as it appeared to be needed for accurate initiation in in vitro assays but not in in vivo assays. McKnight and Kingsbury's latest results suggest that it is required both for accurate initiation and for normal efficiency of transcription in vivo.

The other two segments important for control are a guanine-rich segment between bases −47 and −61 and a cytosine-rich segment between bases −80 and −105. Mutations here markedly reduce the efficiency of transcription.

Somewhat surprisingly, mutations within the region −61 to −80 had no apparent effect. This segment contains the "CCAAT box," which is found in similar positions in the 5′ flanking regions of most eukaryotic genes and is

Science, 23 July, p. 316.

0036-8075/82/0730-0434$01.00/0

considered a strong candidate to be a control sequence. It did not appear to be in this study, however.

McKnight and Kingsbury note that the guanine-rich and cytosine-rich segments contain a six-base pair inverted repeat that could permit the two regions to pair with one another, to form an intrastrand stem-loop structure. They propose that the formation of this structure may somehow facilitate attachment of RNA polymerase II (the transcribing enzyme) onto the *tk* gene control region. This contact would then allow transcription to begin at a specific base.

As yet they do not have direct evidence that the intrastrand looping out occurs and is necessary for transcription initiation, but this should now be easy to test by introducing point mutations that destroy the intrastrand base pairing capacity. If these changes do reduce transcription efficiency, the investigators can try to remedy the situation by introducing, into one gene, complementing mutations that restore the base-pairing.

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Quantum Mechanics Passes Another Test

French photon polarization correlation experiment finds strongest violation yet of Bell's inequality

The success of quantum theory in describing physical phenomena at the molecular level and below is unquestioned. But since 1965 it has been known that quantum mechanics makes certain predictions that are contrary to what is allowed by any member of the class of realistic, local theories. Realism, which to the modern mind might be called common sense, argues that there is an objective reality that exists independent of whether someone observes it or not. Locality stems from the special theory of relativity and its premise that forces or information can only travel between bodies at velocities less than or equal to that of light.

In short, quantum mechanics, special relativity, and realism cannot all be true. Several experiments over the last 10 years strongly support the predictions of quantum mechanics but do not test whether it is special relativity or realism that has to go.

The latest experiment is by Alain Aspect, Philippe Grangier, and Gérard Roger of the Institute of Theoretical and Applied Optics of the University of Paris-South in Orsay (*1*). The investigators measured correlations between the linear polarizations of the two photons emitted when a calcium-40 atom, initially in an excited state with a total angular momentum $J = 0$ decays to its ground state, also with total angular momentum $J = 0$. The decay is by way of an intermediate state with total angular momentum $J = 1$. And the two photons move away from the calcium atom in opposite directions.

What would one expect to see? From the quantum theory as applied to atoms, it is required that the linear polarizations of the photons be parallel. Thus, if polarization analyzers are placed in front of photodetectors (assumed to be 100 percent efficient) and are aligned parallel, either both detectors will register a photon or neither will. If the analyzers are perpendicular, only one of the detectors will register a photon. So far, no problem. The predictions of quantum mechanics and realistic, local theories diverge only when the polarization analyzers assume arbitrary relative orientations.

In 1965, John Bell of the European Laboratory for Particle Physics (CERN) near Geneva published his findings for the case of particles with spin angular momentum of ½. Bell showed that every realistic, local theory of two spin-½ particles obeys a certain inequality that quantum mechanics violates. Subsequently, Bell and several other theorists independently rederived similar inequalities that were appropriate for particular sets of circumstances (*2*).

The inequality tested by the French researchers was found in 1969 by John Clauser, then at Columbia University, Michael Horne and Abner Shimony of Boston University, and Richard Holt, then at Harvard University. The inequality applies to the linear polarization of photons (spin-1 particles) in the situation where an analyzer would pass a photon of polarization either parallel or perpendicular and tell the observer which it was. At any arbitrary relative orientation of the analyzers, four outcomes are possible: both photons are polarized parallel, both are perpendicular, one is parallel and the other is perpendicular, and vice versa. The theorists proved that measurements of a large number of outcomes at each of four relative orientations would have to be such that a certain sum satisfied an inequality. The inequality states that the magnitude of a sum S is less than or equal to 2. A quantum mechanical calculation of the same sum gives the result S less than or equal to $2\sqrt{2}$.

In general, because of photodetectors that are not 100 percent efficient, polarization analyzers that do not perfectly pass or block polarized light, the finite size of optical elements, diverging light beams, and so on, the quantum mechanical sum S can never be achieved. For the French experiment, the quantum mechanical S calculated with these limitations taken into account was 2.70 ± 0.05. The measured value was 2.697 ± 0.015, a rather impressive violation of Bell's inequality. Moreover, according to quantum mechanics, a correlation coefficient E (defined as the sum of the outcomes in which both photons are parallel or perpendicular minus the outcomes in which one photon is parallel and the other perpendicular) has a cosine dependence on the angle between the analyzers. Aspect and his colleagues reproduced this dependence to within 1 percent in measurements at six different orientations.

The sum S is obtained from these correlation coefficients taken at four angles. Broadly speaking, correlation answers the question: How much can one tell about the polarization of one photon from a measurement on the other photon? When the orientations of the polarization analyzers are the same, the correlation is, within experimental error, perfect. In other words, a measurement of only one photon suffices to tell the experimenter what the polarization of the other photon is. At arbitrary angles, the correlation drops, but it is not zero. Realistic, local theories imply a limit, as expressed by Bell's inequality, to how strong the correlation can be. Quantum mechanics says the correlation can be higher.

The source of the correlations lies in

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