8. R. L. Connor, F. B. Mallory, J. R. Landrey, C. W. Iyengar, *J. Biol. Chem.* **244**, 2325 (1969).
9. J. D. Johnston, F. Gautschi, K. Bloch, *J. Biochem.* **224**, 185 (1957).
10. H. E. Vroman and C. F. Cohen, *J. Lipid Res.* **8**, 150 (1967).
11. T. J. Scallen and W. Krueger, *ibid.* **9**, 120 (1968).
12. Relative retention time (to cholestane) on SE-30 column of unesterified desmosterol was 2.05; RRT of impurity was 1.76.
13. M. E. Dempsey, *J. Biol. Chem.* **240**, 4176 (1965).
14. R. Holmes, J. Helms, G. Mercer, *J. Cell Biol.* **42**, 262 (1969).
15. R. Fumagalli, E. Grossi, P. Paoletti, R. Paoletti, *J. Neurochem.* **11**, 561 (1964).
16. E. G. Paoletti, C. R. Sirtori, J. F. Weiss, R. Paoletti, *Advan. Exp. Med. Biol.* **4**, 457 (1968).
17. R. L. Conner, J. R. Landry, C. H. Burns, F. B. Mallory, *J. Protozool.* **15**, 600 (1968).
18. C. G. Mackenzie, J. B. Mackenzie, O. K. Reiss, D. E. Philpott, *Fed. Proc.* **23**, 375 (1964).
19. Supported, in part, by PHS research grant RO1-HE 09103 from the National Heart Institute; NSF grant GB 12133; and PHS fellowship award 1-F3-AM-39642 (to R.C.).

19 June 1970

Pathways through Networks of Branched DNA

Abstract. *Differentiation may be controlled by forks in DNA each having a replicatable protein "switch," stable in either a "left" or a "right" configuration, which determines the path through the DNA network taken by RNA polymerases during transcription. The possibility for dedifferentiation exists, but differentiation could be made irreversible by the exertion of a similar control over parts of the paths through the network taken by DNA polymerases. The concept of bistable switches at DNA branch points can be used to account for antibody variability.*

The problems to be faced in interpreting the large body of data on the structure of antibodies have been clearly stated (*1*). The key observations are that many different variable regions of immunoglobulin polypeptide chains are found in most of the possible combinations with a small number of constant regions and that markers, segregating in a simple Mendelian fashion, can be found in both NH_2-terminal and COOH-terminal regions of some of the chains. In attempting to explain these observations, I find that one essentially simple assumption appears to accommodate all the data and leads to the proposal of a possible mechanism for differentiation.

The primary assumption is that DNA can exist in a branched two-dimensional network, as well as in the conventional one-dimensional form, and that at the branch points there are protein "switches" which can have alternative settings: "left" and "right." A simple molecular mechanism can be suggested for evolving DNA with such permanent branches or detours and for setting the switch points in the left or right configuration. During the transcription of a chromosomal region in a differentiated cell, the RNA polymerases are postulated to traverse the DNA networks, following the pathways determined by the switch settings. A switch would be automatically replicated in its given state, yet under suitable circumstances it could be changed to its alternative state, for example, by allosteric interactions of the switch protein with other proteins or small molecules, including hormones. Dedifferentiation would usually be possible, again through allosteric interactions, but differentiation could be made irreversible if only parts of the DNA network were traversed by DNA polymerases during replication.

The plausibility of the proposal depends on whether simple molecular

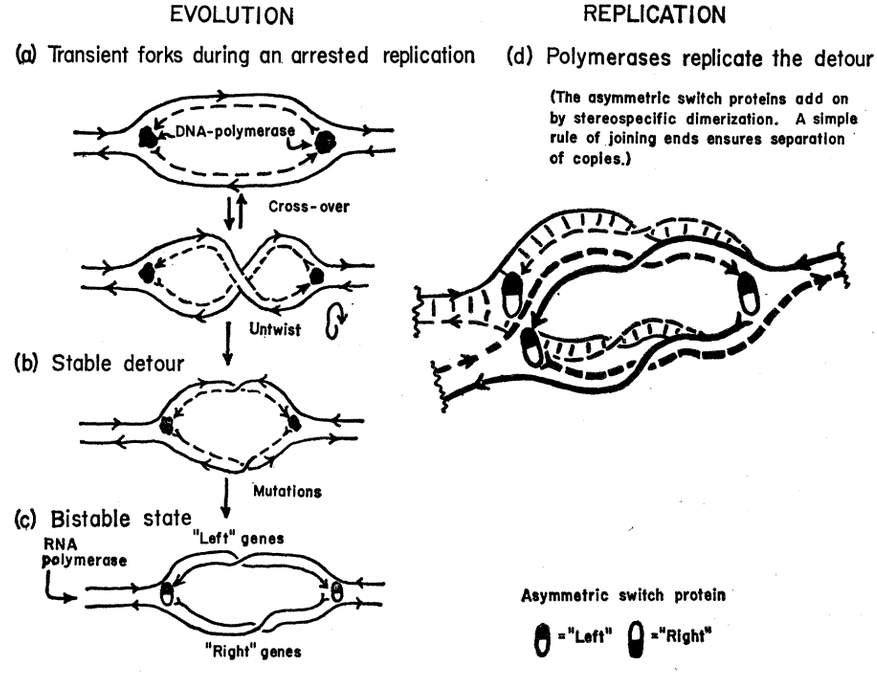

Fig. 1. A conceptual view of the evolution, programming, and replication of a DNA detour. Each line represents a DNA half-helix. The crossover appears to be a necessary prerequisite for a single "joining rule" to yield replicas which could segregate. The figure is not intended to describe the actual events; rather, it illustrates that they are conceptually reasonable.

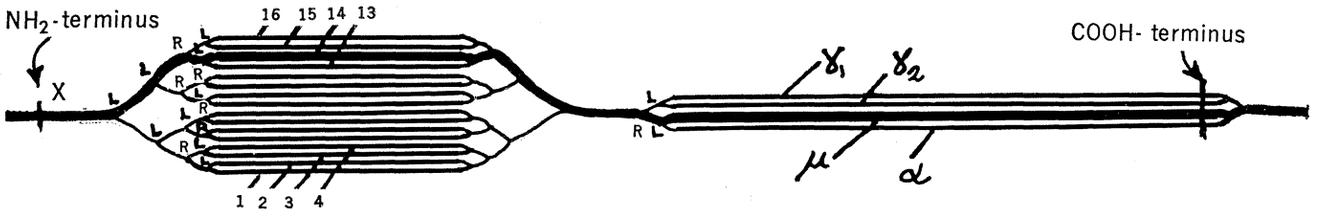

Fig. 2. A hypothetical locus for an immunoglobulin heavy chain with 16 variable regions (V_1 to V_{16}) and four constant parts ($C_{\gamma 1}$, $C_{\gamma 2}$, C_α, C_μ). Each line represents a DNA double helix. Commitment to one of the possible proteins coded by the locus is determined by the random setting of its DNA forks in left (*L*) or right (*R*) configurations; *X* represents a position at which allotypic variants could easily be found. The path of an RNA polymerase molecule is indicated by the heavy line; it would transcribe messenger RNA corresponding to V_{14}–C_μ.

mechanisms can be suggested for the evolution and replication of a permanent DNA detour or fork, for programming the pathway through it, and for replicating the switch setting. Figure 1 attempts to do this. The figure is not intended to be taken too literally; its purpose is simply to demonstrate that all the needed steps are conceivable.

The segregation of branched DNA is compatible with the evolution of detours, branches, and returning loops of DNA in two (but not three) dimensions. Returning loops of DNA branching off linear sections would permit the levels of gene products to be self-regulating. RNA polymerase or DNA polymerase could cycle a loop until the level of a critical product or until the differential gene amplification had reached the height necessary for the loop entrance switch to be changed. DNA networks could thus provide a programmed but essentially unlimited computer-like flexibility for executing the linear instructions contained in their molecules without the need for many diffusible regulatory factors. Flexibility would be enormous with only a modest number of elective branch points or detours, since the maximum number of different paths through a network with n branch points is 2^n (that is, about 10^9 for $n = 30$).

The relevance of the present concept to the problem of antibody variability is illustrated in Fig. 2. The figure portrays the locus for an imaginary immunoglobulin heavy chain which has a genetic marker near its NH_2-terminal end. The protein can have 16 different variable regions (V_1 to V_{16}) and can belong to any of four classes (μ, γ_1, γ_2, or α). Commitment of the cell to the synthesis of only 1 of the 64 possible chains is postulated to be due to a random setting of the left-right switches in the locus; the settings of the switches must be replicated true after the time of commitment. The committed locus shown in Fig. 2 would synthesize mRNA corresponding to V_{14}–$C\mu$. Commitment could be made completely irreversible if the left-right switches controlled the path taken by DNA polymerases through the immunoglobulin locus during DNA replication. I know of no observations in the field of antibody structure (*1*) that are not explicable in terms of loci similar in principle to the one illustrated. There are no obvious reasons why other loci with special features could not show equally or more complex arrangements of lateral multiplicity (*2*).

The concept of programmable branch points in DNA is simple, yet it appears able to explain many aspects of the control of DNA function in complex organisms.

O. SMITHIES

Laboratory of Genetics,
University of Wisconsin, Madison 53706

References and Notes

1. L. Hood and D. W. Talmage [*Science* **168**, 325 (1970)] and F. W. Putnam [*Science* **163**, 633 (1969)] have reviewed the subject of antibody structure and described the basic facts and previous hypotheses.
2. The loci for different tRNA's and for cellular antigens come immediately to mind. F. M. Burnet [*Nature* **226**, 123 (1970)] has suggested that the mechanisms used for generating diversity of cell surface antigens may be similar to those used for antibodies.
3. I thank my colleagues and students for their comments and encouragement, and in particular I thank Dr. D. M. Gibson for stimulating discussions continuing over 3 years and Dr. R. I. DeMars for introducing me to recent papers on chromosomal organization. Supported by NSF GB-4362 and NIH GM-15422. This is paper 1410 from the Genetics Laboratory, University of Wisconsin, Madison 53706.

6 August 1970

Prostaglandin Receptor Site: Evidence for an Essential Role in the Action of Luteinizing Hormone

Abstract. *A dose-response relation was established between prostaglandins and formation of adenosine 3′,5′-monophosphate in the mouse ovary. The prostaglandin antagonist, 7-oxa-13-prostynoic acid, blocked the stimulatory effect of prostaglandin E_1, prostaglandin E_2, and luteinizing hormone on adenosine 3′,5′-monophosphate formation in a competitive manner. Kinetic studies made it possible to suggest that there is a single luteinizing-hormone-related prostaglandin receptor in mouse ovaries, and that activation of this prostaglandin receptor is an essential requirement in the action of luteinizing hormone to stimulate adenosine 3′,5′-monophosphate formation and steroidogenesis.*

The suggestion that prostaglandins (*1*) may regulate intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP), the postulated second messenger of hormone action (*2*), was obtained mainly from studies on adipose tissue in which both the efflux of prostaglandin (*3*) and the formation of cyclic AMP were shown to increase as a result of hormonal stimulation. Since prostaglandins inhibit hormonally induced cyclic AMP formation in adipose tissue (*4*) as well as in isolated lipocytes (*5, 6*) a negative feedback role was assigned to these substances, as outlined by Horton (*7*). A similar negative feedback mechanism also appears applicable in the case of the action of vasopressin on kidney tubules (*8*) and gastrin (*9*) on the rat stomach mucosa. However, in all other tissues studied (*5*), prostaglandin E_1 (PGE_1; letters and subscripts are used to differentiate prostaglandins) was found to stimulate the formation of cyclic AMP and thus mimic rather than antagonize the actions of the individual hormones. Measurements in vitro (*10*) with rat adrenals, in which prostaglandins and cyclic AMP were found to duplicate the effect of adrenocorticotrophic hormone (ACTH) in increasing steroidogenesis, led to the suggestion that prostaglandins serve a positive as well as negative feedback role in the action of hormones. In addition, it has been postulated (*11*) that $PGF_{2\alpha}$ and luteinizing hormone (LH) work through the same mechanism to increase progesterone synthesis in rat ovaries. To our knowledge, however, there has been no evidence presented to suggest that the prostaglandins may play an essential role in the action of hormones to stimulate adenyl cyclase, as is indicated from data presented in this report.

Female Charles River mice (60 to 70 days of age) were killed by cervical dislocation, and their ovaries were excised and denuded of surrounding fat. Three intact ovaries were first incubated at 37°C for 1 hour in 2 ml of Krebs-Ringer phosphate buffer, *p*H 7.2 (*12*), with only one-half of the prescribed Ca^{2+} concentration and containing 6 m*M* glucose and 1 μc of purified [8-^{14}C]adenine. After this incubation, which brought about formation of intracellular [^{14}C]adenosine triphosphate (ATP), theophylline was added to all tubes to a final concentration of 5 m*M*. The prostaglandins and gonadotropin were then added, and the incubation was continued for 30 minutes. The reactions were terminated by the addition of 0.4 ml of 10 percent trichloroacetic acid, and cyclic [^{14}C]-AMP was subsequently isolated and measured, with paper chromatography being the final step, as described (*13*).

Our studies revealed that, as with