Fox

April 30, 1959

Dr. Katherine Wilson, Executive Secretary
Genetics Study Section
Division of Research Grants
National Institutes of Health
Bethesda 14, Maryland

In re: C-2440 (C4S1)

Dear Dr. Wilson:

In response to your request, the following is a summary report of progress achieved by the above project during the past two years. Only major points are included.

The work of the project has centered around the problems of the genetic control of protein synthesis. From a genetic point-of-view, the most important problems of gene-to-protein information transfer are those of the determination of amino acid sequence and the control of folding of the polypeptide chain. From a biochemical point-of-view, the problems consist of demonstrating the involvement of the biochemical processes involved in protein synthesis, i.e., activation of amino acids by ATP, transfer of amino acids to and transport by "soluble" RNA, condensation of amino acids into a polypeptide chain presumably on a microsomal RNA template, removal of the polypeptide chain from the RNA surface, and formation of foldings and cross-linkages.

The genetic control of amino acid sequence has been demonstrated by Ingraham in human hemoglobin. Our own work has dealt with two cases which apparently involve the genetic control of folding, i.e. the "gross" or "tertiary" configuration of the protein molecule.

The first of these analyses has dealt with the enzyme tyrosinase in Neurospora crassa. In previous work, preliminary genetic analysis of the difference in tyrosinase activity between the mating types of strain 15300 (albino-2) had suggested that enzyme concentration and enzyme specificity are controlled by two different genetic systems, both probably complex and neither involving the mating type locus. This genetic duality led to the formulation of a tentative hypothesis regarding tyrosinase synthesis (Fox, 1954, Nature 173:350-351):

$$\xrightarrow[\text{Stage 1}]{} \text{protyrosinase} \xrightarrow[\text{Stage 2}]{} \text{tyrosinase}$$

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It was proposed that each of these stages is under genetic control. Stage 1 could involve establishment of amino acid sequence and preliminary folding, while stage 2 could involve establishment of final tertiary configuration.

The major features of this proposal have now been validated (Fox and Burnett, 1959). Protyrosinase, a large, enzymatically inactive polypeptide, electrophoretically separable from tyrosinase, has been demonstrated and isolated. In mycelial extracts it is activated to form tyrosinase of the same specificity as is formed in vivo. The kinetics of activation are first order, suggesting a monomolecular or pseudomonomolecular process. Activation is mediated by one or more enzymes, electrophoretically separable from both protyrosinase and tyrosinase. Cultures which are genetically incapable of forming tyrosinase either fail to form protyrosinase or fail to form activating enzyme.

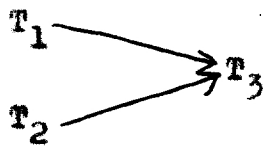
These results are in marked contrast to those reported by Horowitz and Fling. These workers report a pair of contrasting alleles, T^s and T^l, producing respectively thermostabile and thermolabile tyrosinase. Since only one of the two enzymes could be found in a homocaryon (depending on which allele was present), and since both enzymes were produced in unaltered form in heterocaryons, it was concluded that each of the alleles produces its respective enzyme without interaction, i.e. by means of a template mechanism. No differences in specificity (Michaelis constant, turnover number) could be found between the thermostable and thermolabile enzymes, and strip paper electrophoresis yielded no evidence of heterogeneity in either case.

Until recently, the connection between the analysis of Horowitz and Fling and our own was not apparent. We had also been unable to demonstrate heterogeneity in strip paper electrophoresis, but it appeared that only the thermostabile tyrosinase was present in our material. We now find, however, that continuous paper electrophoresis serves to separate the tyrosinase extracted from homocaryons into three components of indistinguishable specificity. One of these (T_1) exhibits an energy of thermal inactivation of 95,800 cal./mole, virtually identical with the value reported by Horowitz and Fling for their thermolabile tyrosinase. The other two (T_2 and T_3) exhibit energies of inactivation equal to 60,900 and 57,300 cal./mole respectively, closely similar to the values reported by Horowitz and Fling for their thermostabile enzyme. Together, T_2 and T_3 constitute 96% of the tyrosinase extracted. The presence of T_1 cannot be detected without prior fractionation by continuous paper electrophoresis. Moreover, during incubation of crude extracts at low temperatures (5°-25°), T_1 and T_2 disappear. Quantitative measurements disclose that the decrease in T_1 and T_2 is exactly compensated by an increase

in T_3, as if both of the former are converted into the latter:

$$(T_1 \longrightarrow T_2) \longrightarrow T_3$$

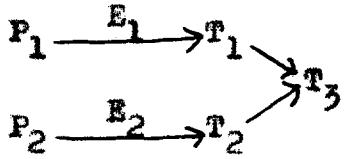
or



Overall, the terminal stages of tyrosinase synthesis seem to be as follows:

$$P \xrightarrow{E} (T_1 \longrightarrow T_2) \longrightarrow T_3$$

or



(P, protyrosinase; E, activating enzyme or enzymes; T_1, "thermolabile" tyrosinase; T_2 and T_3, "thermostabile" tyrosinase.)

It is entirely possible that the conversion of T_1 and T_2 to T_3 is also enzymatically catalyzed.

The identical specificities of T_1, T_2, and T_3 would seem to render the first of these schemes preferable, and would indicate that specificity is determined in earlier stages of synthesis, i.e. at the time of determination of amino acid sequence or during preliminary folding. The differences in thermostability and electrophoretic mobility of T_1, T_2, and T_3 suggest differences in tertiary structure rather than in their active centers. The conversion of the two former into the latter suggests that the T locus of Horowitz and Fling produces its effects by controling the rate of these terminal stages in tyrosinase synthesis, thus leading to the predominance of one or the other of the enzymes, rather than by means of a template mechanism. (These results will be reported at the forthcoming A~~A~~. I. B. S. meetings.)

The second of the two cases which we have investigated has dealt with antigenic effects of the sex chromosomes in Drosophila melanogaster (Fox and Yoon, 1958; Fox, 1958; Fox, 1959). Changes in X-chromosome dosage result in qualitative shifts in antigen pattern, as is summarized in

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the following table.

Sex Chromosomes	Antigens			
	♂-1	♂-2	♀-1	♀-2
X/X	-	-	+	+
XX/Y	-	-	+	+
$X \cdot Y^S/X \cdot Y^S$	-	-	+	+
$X \cdot Y^L/X \cdot Y^L$	-	-	+	+
X/X·Y	-	-	+	+
X/Y	+	+	-	-
X/O	+	+	-	-
$X \cdot Y^S/Y$	+	+	-	-
$X \cdot Y^L/Y$	+	+	-	-
X·Y/O	+	+	-	-

Each of the four antigens is a protein and possesses distinct antigenic specificity - there is no cross-reaction of one with antibody to any of the others. As may be seen, individuals with one X-chromosome, regardless of the presence or absence of all or part of the Y, possess antigens ♂-1 and ♂-2. Individuals with two X-chromosomes, regardless of Y constitution, possess ♀-1 and ♀-2. The Y chromosome is thus not involved, and the effect is attributable to the difference in dosage of one or more euchromatic loci on the X, possibly the sex-determining loci themselves.

The effect is obviously one on the configuration of the antigenic groupings themselves. The significance of the existence of two specific antigens in each case is not yet understood, but they may be true alternatives. This is another instance of an effect of euchromatic loci on the antigenic specificity of proteins, but since it is a qualitative effect of dosage it is improbable that a simple template mechanism is involved. It seems likely that the differences involve either differences in amino acid sequence or in preliminary folding (or perhaps in both).

The heterochromatic Y-chromosome has no detectable effect on the antigenic specificity of proteins. However, the immunological and physical properties of a particular protein are subject to a maternal influence of the Y-chromosome.

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When a female lacks a Y-chromosome (X/X) her progeny exhibit an antigenic specificity (Y-1) associated with a protein capable of inducing antibody formation in rabbits, and capable of uniting with and precipitating the specific antibody. The term "complete" is used to describe the properties of the antigen under these conditions. When a female possesses a Y-chromosome ($\overline{XX}$/Y) her progeny exhibit the same antigenic specificity (Y-1), but it is associated with a protein which is incapable of precipitating the specific antibody, although it is capable of inducing formation of the antibody and of uniting (inhibiting) with it *in vitro*. The term "incomplete" is applied to this situation. The effect seems to be localized in the proximal portion of the short arm of the Y-chromosome, *i.e.* to that portion carrying the nucleolar organizer.

The difference between complete and incomplete Y-1 probably reflects a physical difference between the respective molecules of a sort not involving a difference of their antigenic groupings but rather in their number. Both forms of Y-1 are non-dialyzable, heat labile, and precipitated by protein precipitants. Complete Y-1 is precipitated from crude extracts by simple dialysis against water. A physical difference between complete and incomplete Y-1 is suggested by a greater resistance of the former to inactivation during lyophilization. The two forms could differ either in the size of their respective molecules or in the way in which the polypeptide chain is folded.

It is interesting to note that it is the presence of the Y-chromosome in the oocyte of a female that is responsible for this effect, but that the effect is observed in her progeny as adults. The loss of the Y during meiosis does not alter the result. Thus, a self-perpetuating mechanism must be established in the oocyte, capable of maintaining itself in the soma during all of development.

These observations are probably associated with those of Schultz and coworkers that the Y-chromosome influences the base constitution of RNA synthesized in the oocyte. Since euchromatic changes have effects on specificity, while heterochromatic changes seem to affect only tertiary structure, it is possible to suggest that each produces an RNA of different function. Euchromatin may produce RNA concerned with the determination of amino acid sequence and preliminary folding, while heterochromatin might produce RNA concerned with the determination of final tertiary structure. Both of these kinds of RNA could be the constituents of the cytoplasmic (microsomal) ribonucleoprotein particles, and some kind of mechanism of somatic self-perpetuation is conceivable.

These observations and speculations serve to indicate the direction of future work and provide a connection with

the recent biochemical developments referred to above.

The following publications have resulted from the work of the project during the past two years:

Fox, A. S. 1957. Genes. The Encylopedia of Chemistry, G. L. Clark and G. G. Hawley, eds., Reinhold, New York, pp. 442-444.

Fox, A. S., and S. B. Yoon. 1957. Application of agar-diffusion techniques to the analysis of Drosophila antigens. Anat. Rec. 128:552 (Abstract).

Fox, A. S., and S. B. Yoon. 1957. Genetic mechanisms responsible for antigenic differences between males and females in Drosophila melanogaster. Genetics 42: 370 (Abstract).

Fox, A. S., and J. B. Burnett. 1957. The components of the protyrosinase activation system in Neurospora crassa, strain 15300, and their production by genetically different cultures. Genetics 42:370 (Abstract).

Fuscaldo, K. E. 1957. A technique for the collection of bacteriologically sterile flies. Drosophila Information Service 31:174-175.

Mead, C. G. 1957. The effect of Bar, Enhancer of Bar, and changes in their positions on the free amino acids and peptides of D. melanogaster. Drosophila Information Service 31:133-134.

Fox, A. S., and S. B. Yoon. 1958. Antigenic differences between males and females in Drosophila not attributable to the Y chromosome. Transplant. Bull. 5:52-55.

Fox, A. S. 1958. The genetics of tissue specificity. Transplant. Bull. 5:77 (Abstract).

Fox, A. S., and J. B. Burnett. 1958. Kinetics of tyrosine oxidation by crude tyrosinase preparations from Neurospora crassa. Proc. Soc. Exp. Biol. Med. 98:110-114.

Fox, A. S. 1958. Immunogenetic studies in relation to problems of protein synthesis. Proc. X Int. Cong. Gen. 2:84-85 (Abstract).

Fox, A. S. 1958. Genetics of tissue specificity. Annals N. Y. Acad. Sci. 73:611-634.

Fox, A. S., and J. B. Burnett. 1959. The genetics and biochemistry of tyrosinase in Neurospora crassa. In Pigment Cell Biology, M. Gordon ed., Academic Press, pp. 249-277.

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Fox, A. S., Mead, C. G., and I. L. Kunyon. 1959. The sex peptide of Drosophila melanogaster. Science, in press.

Fox, A. S. 1959. Genetic determination of sex-specific antigens. Jour. Nat. Cancer Inst., in press.

Sincerely yours,

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