Appendix 1: Texts of Proposal Summaries

Dr. Bloch's Abstract

Version 1

1. A search is being conducted for sequence homologies and for homologies of the reverse complementary sequences among tRNAs and rRNAs. 2. The results of these searches are being compiled in order to compare the distributions of the sequences among different regions of the molecules.

3. The purpose is to search for evidence of common origins of these classes of RNA.

4. A model is proposed for the evolutionary origin of the protein synthetic mechanism which predicts a common origin of the different classes of RNA. 5. The model is based on a synthesis of a multifunctional RNA through a series of alternating syntheses: elongation through looping back, replication via templating, then repetition of the this cycle [sic], starting with a primoridal tRNA with a simple anticodon region. 6. The result would be a molecule with extensive internal complementarity, dotted with codons and anticodons, capable of assuming configurations that would permit it to serve as message and structural RNA, and alternatively as gene.

7. The model predicts extensive homologies between the primordial tRNA and rRNAs. 8. Homologous sequences in present day tRNAs and rRNAs are being found.

9. An attempt is being made to sort out the relative importance of function and common descent as explanations for the homologies, by studies of the commonality of the homologies and their placements within and among the RNAs of the different classes.

Version 2

1. Ribosomal RNA is peppered with tracts that are homologous with regions found among the different transfer RNAs. 2. The matches are too frequent and extensive to be attributed to coincidence. 3. Their distributions and patterns suggest a common evolutionary origin for the two classes of molecules. 4. Function as an explanation for their existence

appears unlikely but cannot be ruled out. 5. Different domains have been conserved in different classes of organisms. 6. Our work will continue to identify examples of these homologies by searching for them among a variety of organisms. 7. The search was prompted by a model for the origin of a primitive multifunctional RNA molecule. 8. In the model, a short RNA with a codon or anticodon near the 3′ end undergoes successive rounds of elongation by self-priming (looping back) and self-templating, giving rise to an RNA in which codons are held in contiguous configurations by secondary folding. 9. The subsequent split of message, transfer, and ribosome functions is thought to follow acquisition of the cellular habit. 10. The model suggested the existence of homologies among present day t- and r-RNAs and this prediction is being realized. 11. The interpretation of the homologies is of importance. 12. A multidimensional test for evolutionary convergence has been designed and is being used to determine whether the homologies do indeed reflect common origin rather than function. 13. Filling out the rRNA map, through continued accumulation of homologies, should permit the reconstruction of a primordial RNA molecule.

Version 3

1. A large minority of tRNAs from all species of organisms studied have stretches whose base sequences are identical or nearly so to stretches found in rRNAs.

2. They are too frequent and too extensive to be attributed to coincidence.

3. Factors contributing to these matches might be shared functions at the RNA or DNA levels, or common origins. 4. The latter might be of recent derivation through recombination and transfection, or relics of ancient origin. 5. The matching sequences are distributed without discernable pattern among the molecules and among species. 6. Their frequent appearance, often unique to interspecies comparisons, indicates that they need not result from selection for interaction in a common cellular environment. 7. They are also thought to be conserved vestiges of ancient origin. 8. The occurrence of overlapping sets of homologies within species, and confirming overlays among species (homologies found in independent searches in different organisms that occupy equivalent positions on the rRNAs, and assign similar base sequences) suggest that their continued identification should permit the reconstruction of an RNA that is ancestral to both tRNAs and rRNAs. 9. Such a "synthesis" should help to provide an understanding of the early evolution and current functions of the transcription–translation mechanisms.

Dr. Crews's "Specific Aims"

Version 1

I propose to work in the area of reproductive biology, concentrating on the regulation of reproduction by internal and external stimuli in seasonally breeding vertebrates. Specifically, I will continue my studies of two reptile species that differ markedly in their reproductive physiology.

The green anole lizard is similar to many laboratory and domesticated mammals and birds in that the peak gonadal activity (gamete maturation accompanied by a substantial increase in the circulating level of sex steroid hormones) is *associated* with mating. Species that exhibit such a reproductive tactic frequently have a functional association between sex hormones and sexual behavior. Previous research with the green anole lizard has shed new light on ecological and evolutionary adaptations of the neuroendocrine mechanisms controlling sexual behavior and reproductive physiology. In contrast, the red-sided garter snake, as well as many other vertebrates including some mammals, exhibits a *dissociated* reproductive tactic. In these species, production of gametes and maximal sex hormone secretion are temporally dissociated from mating behavior. In the garter snake, gametes are produced in late summer only after the breeding season is ended; the gametes are then stored until the next mating period. Thus, unlike those species with associated reproductive tactics, mating in the red-sided garter snake occurs when the gonads are completely regressed and circulating levels of sex hormones are low. This implies that the causal mechanisms of mating behavior, at least at the physiological level, must be fundamentally different in species with dissociated reproductive tactics. Recent studies of the red-sided garter snake indicate that this is the case.

The observation that gonadal and behavioral cycles can be dissociated is itself not new, but the implications of this observation have not been fully appreciated. I present here a systematic and comparative series of studies that will focus on specific questions involving the causal mechanisms and functional outcomes of sexual behavior in these two species. From this comparison will emerge a new perspective on the many species, life histories, and sex differences observed in vertebrates. In addition to contributing to our understanding of related areas of reproductive biology, including gamete storage and animal husbandry, this research will yield insight into fundamental reproductive processes.

Version 2

In general I am interested in the biopsychology of reproduction, or more precisely the regulation of reproduction by internal and external stimuli in seasonally breeding invertebrates. The general objectives of my research are to i) investigate how the environment regulates reproduction, ii) determine how reproductively relevant stimuli are perceived and integrated in the central nervous system, iii) demonstrate how the information regulates internal reproductive state, and iv) examine how changes in internal state influence the expression of behavior. To this end, I use a comparative approach that combines and integrates the physiological, morphological, organismal, and ecological levels of analysis. The emphasis on laboratory and field experiments reveals the causal mechanisms and functional outcomes of reproductive behavior on each level without obscuring the relations among the levels. Moreover, the laboratory and field studies are complementary. The field has proven to be a valuable testing ground for hypotheses; similarly, the laboratory is the only possible arena for determining the physiological bases of phenomena observed in the field.

The specific objective is to examine the causal mechanisms and functional outcomes of the two major annual reproductive tactics—associated and dissociated—exhibited by higher vertebrates. In many seasonally breeding vertebrates, gamete production and maximum secretion of sex steroid hormones precedes immediately or coincides with courtship and copulatory (mating) behavior. This annual pattern may be termed the *associated* reproductive tactic, or prenuptial gametogenesis (Figure 1). A markedly different annual pattern is exhibited in many vertebrates, including some mammals, in which the gametes are produced only after the breeding season has ended; the gametes are then stored until the next breeding period. In these species, mating occurs when the gonads are not producing gametes and blood levels of sex steroid hormones are basal. This pattern may be referred to as a *dissociated* reproductive tactic, or postnuptial gametogenesis (figure 1 [Crews's figure no.]).

I will focus on one representative species of each reproductive tactic. The green anole lizard is similar to many laboratory and domesticated mammals and birds in showing the associated tactic. In contrast, the red-sided garter snake shows the dissociated pattern. In many instances a direct comparison of these two species will be made, whereas in other instances gaps in our knowledge must be filled before conceptually valid comparisons can be made. Thus, some of the proposed experiments deal only with one species or tactic. Ultimately, however, my goal is to compare the two tactics at as many levels of organization as are feasible and reasonable. Such a broad approach is crucial if important generalities

underlying reproductive processes are ever to emerge. My proposed studies will contribute directly to our understanding of related areas of reproductive biology, including gamete storage and animal husbandry.