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LIVING OÖCYTE of the South African clawed frog *Xenopus laevis* is microinjected with a foreign messenger RNA. The messenger codes for rabbit globin, and the unspecialized oöcyte, a precursor of the frog egg, will synthesize rabbit hemoglobin, which is ordinarily produced only in specialized red blood cells of a very different animal. The large, bicolored oöcytes are about a millimeter in diameter; the smaller pale cells are immature oöcytes. The sharpened glass needle (*top left*) with a tip about 20 micrometers (thousandths of a millimeter) in diameter, connected by oil-filled tubing to a syringe, can accurately deliver volumes of fluid as small as 10 nanoliters (billionths of a liter), and it is possible to inject more than 200 oöcytes an hour. The photomicrograph was made by the author and Neil Papworth.

Rabbit Hemoglobin from Frog Eggs

Messenger RNA from a specialized cell of one species is translated by the egg of another species. Such molecular biology experiments, done in living cells, give information on control of gene expression

by Charles Lane

What happens if a frog egg is given instructions, in the form of a particular molecule of the nucleic acid RNA, to make rabbit hemoglobin? The egg makes rabbit hemoglobin. What if the frog egg is supplied with the RNA molecule that specifies honeybee venom? It makes honeybee venom. Such results are not merely bizarre biochemical anomalies. They provide significant information about the expression of particular genes in particular cells. The control of gene expression is central to normal animal development and to such abnormal processes as cancer, birth defects and metabolic disorders of genetic origin. The experiments I shall describe are attempts to gain an understanding of that control by doing molecular biology in living cells. We inject various components of differentiated cells back into embryonic cells in order to study the molecular mechanisms involved in development.

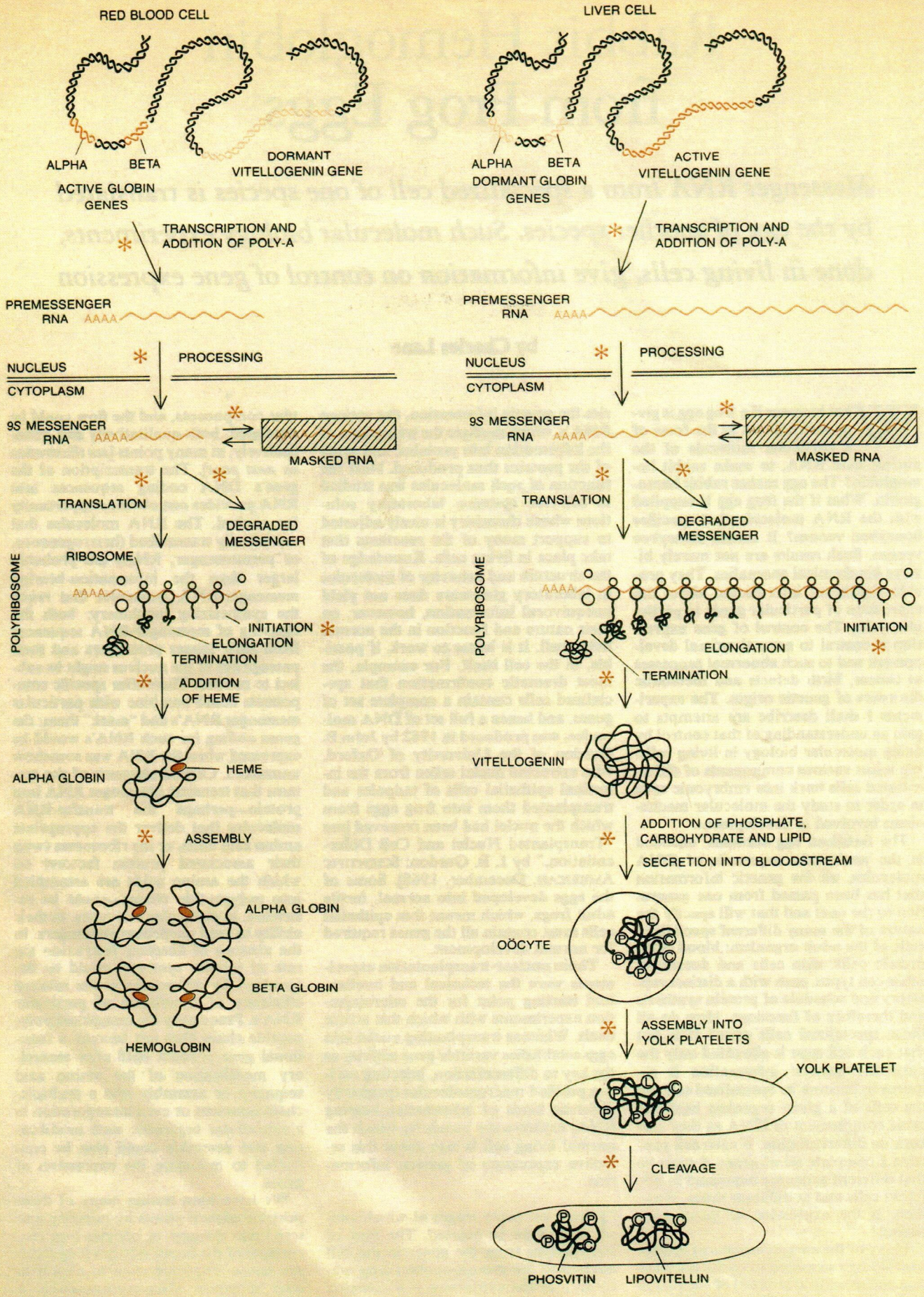
The fertilized egg contains, encoded in the nucleotide sequences of DNA molecules, all the genetic information that has been passed from one generation to the next and that will specify the nature of the many different specialized cells of the adult organism: blood cells, muscle cells, skin cells and dozens of other cell types, each with a distinct repertory and schedule of protein synthesis and therefore of functions. How do all those specialized cells arise? It is not that each cell type is allocated only the particular genetic information it requires to become its specialized self; all the cells of a given organism have the same complement of DNA as they embark on differentiation. If each cell contains a complete set of genes, it must be that different genes are expressed in different cells and at different times. How, then, is the expression of genes controlled?

Many of the early triumphs of molecular biology stemmed from the purification and structural analysis of such large molecules as those of DNA, which carries the genetic information, the various RNA's, which mediate the translation of the information into proteins, and some of the proteins thus produced. Next the function of such molecules was studied in cell-free systems: laboratory solutions whose chemistry is nicely adjusted to support many of the reactions that take place in living cells. Knowledge of the structure and behavior of molecules in laboratory glassware does not yield unequivocal information, however, on their nature and function in the normal living cell. It is better to work, if possible, in the cell itself. For example, the most dramatic confirmation that specialized cells contain a complete set of genes, and hence a full set of DNA molecules, was produced in 1962 by John B. Gurdon of the University of Oxford, who extracted nuclei taken from the intestinal epithelial cells of tadpoles and transplanted them into frog eggs from which the nuclei had been removed [see "Transplanted Nuclei and Cell Differentiation," by J. B. Gurdon; SCIENTIFIC AMERICAN, December, 1968]. Some of the eggs developed into normal, fertile adult frogs, which meant that epithelial cells must contain all the genes required for normal development.

Those nuclear-transplantation experiments were the technical and intellectual starting point for the microinjection experiments with which this article deals. Whereas transplanting nuclei into eggs establishes variable gene activity as the key to differentiation, injecting various purified macromolecules (primarily different kinds of information-bearing RNA) explores the means by which the normal living cell brings about this selective expression of genetic information.

There are many stages at which control might be exerted. The flow of information from the genes in the cell nucleus to the protein-synthesizing machinery in the cytoplasm is a complex process involving a multitude of molecular components, and the flow could be regulated, both qualitatively and quantitatively, at many points [*see illustration on next page*]. The transcription of the gene's DNA coding sequences into RNA provides one obvious opportunity for control. The RNA molecules that are initially transcribed (heterogeneous, or premessenger, RNA) are probably larger than the information-bearing messenger-RNA molecules that reach the synthesizing machinery; both the cleaving of messenger-RNA sequences from their longer precursors and their passage out of the nucleus might be subject to control. Thereafter specific components could combine with particular messenger RNA's and "mask" them; the genes coding for such RNA's would be expressed when the RNA was somehow unmasked. Certain elements of the systems that translate messenger RNA into protein—perhaps the transfer-RNA molecules that deliver the appropriate amino acid units, or the ribosomes (with their associated protein factors) on which the amino acids are assembled into polypeptide chains—could be restricted, in particular cell types, in their ability to read particular messengers. In the absence of absolute restriction the rate of protein synthesis could be determined by differences in the relative efficiency of translation of particular RNA's. Frequently the completed polypeptide chain does not become a functional gene product until after secondary modification of the amino acid sequence or assembly into a multiple-chain structure or even incorporation in a subcellular organelle; such modification and assembly could also be controlled to modulate the expression of genes.

We have been testing many of these possible control points by injecting material into the eggs or oöcytes (egg precursors) of the South African frog *Xenopus laevis*. They are easy to obtain in large numbers. They are enormous; a fully grown oöcyte, just before it is


RED BLOOD CELL
ALPHA
BETA
ACTIVE GLOBIN GENES
DORMANT VITELLOGENIN GENE
TRANSCRIPTION AND ADDITION OF POLY-A
PREMESSENGER RNA
AAAA
NUCLEUS
CYTOPLASM
PROCESSING
9S MESSENGER RNA
MASKED RNA
TRANSLATION
DEGRADED MESSENGER
POLYRIBOSOME
RIBOSOME
INITIATION
ELONGATION
TERMINATION
ADDITION OF HEME
ALPHA GLOBIN
HEME
ASSEMBLY
BETA GLOBIN
HEMOGLOBIN
LIVER CELL
ALPHA
BETA
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MASKED RNA
TRANSLATION
DEGRADED MESSENGER
POLYRIBOSOME
INITIATION
ELONGATION
TERMINATION
VITELLOGENIN
ADDITION OF PHOSPHATE, CARBOHYDRATE AND LIPID
SECRETION INTO BLOODSTREAM
OÖCYTE
ASSEMBLY INTO YOLK PLATELETS
YOLK PLATELET
CLEAVAGE
PHOSVITIN
LIPOVITELLIN

transformed by a hormonal stimulus into an egg, is more than a millimeter in diameter, some 10,000 times larger than a frog liver cell. And it is resilient enough to withstand the injection of an amount of fluid equal to 5 percent of its volume.

In 1963 Gerard Marbaix, A. Burny and H. Chantrenne of the Free University of Brussels purified an RNA species they believed to be the messenger specifying globin, the protein component of hemoglobin. It was the expected size (nine Svedberg units) and was found linking polyribosomes, or groups of ribosomes, in rabbit reticulocytes: immature red blood cells that make hemoglobin and little else. Six years later the Belgian workers had still not been able to demonstrate biological activity in their 9*S* fraction, but that year Raymond E. Lockard and Jerry B. Lingrel of the University of Cincinnati managed to translate a similar 9*S* fraction in a cell-free system, a lysate of red blood cells. At that time Gurdon and I were experimenting with the injection into oöcytes of reticulocyte polyribosomes. Marbaix joined our laboratory at Oxford, and we undertook to inject the purified 9*S* fraction.

We dissolved the 9*S* RNA in a simple buffer solution and injected about 50 nanoliters (billionths of a liter) into each of 20 oöcytes. The oöcytes were incubated overnight with a radioactively labeled amino acid. Then an extract of the oöcytes, to which unlabeled rabbit hemoglobin had been added as a marker, was passed through a column of Sephadex, which separates molecules according to size. The same things were done with control oöcytes, which had been injected with buffer lacking globin RNA. The extract from RNA-treated oöcytes turned out to contain radioactive molecules that were in the same Sephadex fraction—were the same size—as the unlabeled rabbit hemoglobin molecules (which were identified by their color); the extract of the control oöcytes did not contain those radioactive molecules [*see illustrations on next two pages*]. In other words, the RNA-treated oöcytes appeared to be synthesizing spectacular amounts of hemoglobin.

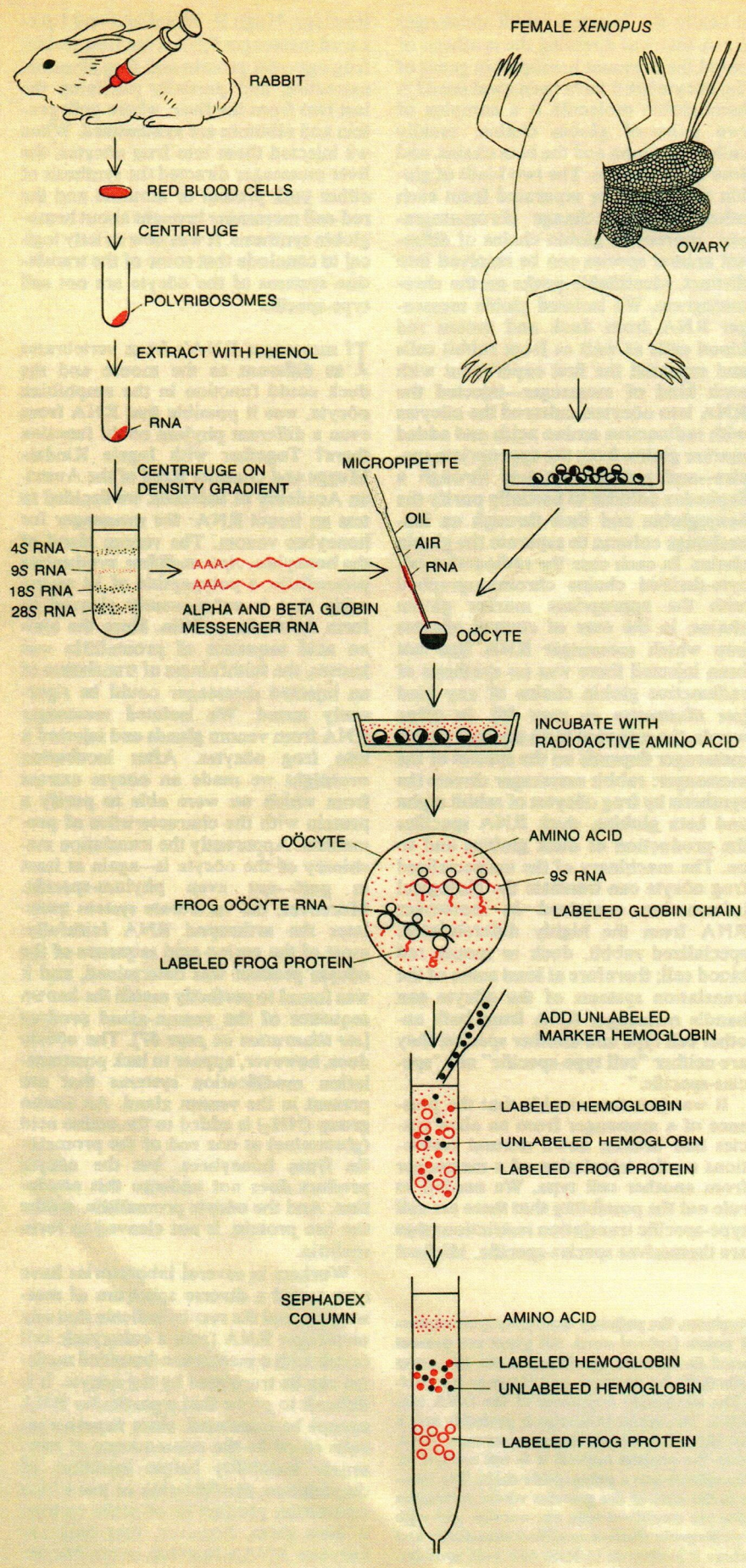
What kind of hemoglobin was it? Was it really the injected rabbit messenger RNA that was directing the synthesis or could the dormant hemoglobin genes of the oöcyte itself have been awakened? A hemoglobin molecule is a complex of two pairs of globin chains, usually called the alpha and the beta chains, and four heme groups. The two kinds of globin chains can be separated from each other by ion-exchange chromatography; moreover, globin chains of different animal species can be resolved into distinct, identifiable peaks on the chromatogram. We isolated globin messenger RNA from duck and mouse red blood cells as well as from rabbit cells and repeated the first experiment with each kind of messenger—injected the RNA into oöcytes, cultured the oöcytes with radioactive amino acids and added marker globin from the appropriate species—and put the extracts through a Sephadex column to partially purify the hemoglobin and then through an ion-exchange column to separate the globin chains. In each case the radioactive oöcyte-derived chains chromatographed with the appropriate marker globin chains; in the case of control oöcytes into which messenger RNA had not been injected there was no synthesis of radioactive globin chains of any kind [*see illustration on page 66*]. In other words, the response to an injected globin messenger depends on the species of the messenger: rabbit messenger directs the synthesis by frog oöcytes of rabbit alpha and beta globins, duck RNA specifies the production of duck globins and so on. The machinery of the unspecialized frog oöcyte can translate the biological information contained in messenger RNA from the highly differentiated, specialized rabbit, duck or mouse red blood cell; therefore at least some of the translation systems of the oöcyte can handle messenger RNA from both another cell type and another species; they are neither "cell type-specific" nor "species-specific."

It was just conceivable that the presence of a messenger from an alien species had broken down normal restrictions on the translation of a messenger from another cell type. We needed to rule out the possibility that there are cell type-specific translation restrictions that are themselves species-specific. Michael Berridge, Hugh R. Woodland and I prepared messenger RNA's for frog globin, frog egg-yolk protein and frog albumin, extracting and partially purifying the last two from the liver, where yolk protein and albumin are synthesized. When we injected them into frog oöcytes, the liver messenger directed the synthesis of either yolk protein or albumin and the red-cell messenger brought about hemoglobin synthesis. It was now strictly logical to conclude that some of the translation systems of the oöcyte are not cell type-specific.

If messenger RNA's from vertebrates as different as the mouse and the duck could function in the amphibian oöcyte, was it possible that RNA from even a different phylum could function there? Together with Ingela Kindas-Mugge and Gunther Kreil of the Austrian Academy of Sciences, we decided to test an insect RNA: the messenger for honeybee venom. The venom gland of the honeybee, *Apis mellifera*, synthesizes promelittin, a polypeptide of 34 amino acids that is subsequently cleaved to form the toxin melittin. Since the amino acid sequence of promelittin was known, the faithfulness of translation of an injected messenger could be rigorously tested. We isolated messenger RNA from venom glands and injected it into frog oöcytes. After incubation overnight we made an oöcyte extract from which we were able to purify a protein with the characteristics of promelittin. Apparently the translation machinery of the oöcyte is—again at least in part—not even phylum-specific. Moreover, the vertebrate system translates the arthropod RNA faithfully: most of the amino acid sequence of the oöcyte product was determined, and it was found to perfectly match the known sequence of the venom-gland product [*see illustration on page 67*]. The oöcyte does, however, appear to lack posttranslation modification systems that are present in the venom gland. An amino group (NH_2) is added to the amino acid (glutamine) at one end of the promelittin from honeybees, but the oöcyte product does not undergo this amidation. And the oöcyte promelittin, unlike the bee protein, is not cleaved to form melittin.

Workers in several laboratories have now tested a diverse spectrum of messengers, and the results indicate that any messenger RNA from a eukaryotic cell (a cell with a membrane-bounded nucleus) can be translated by the oöcyte. It is difficult to prove that a particular RNA cannot be translated, since negative results could be the consequence of messenger instability before injection, of degradation, modification or loss of the translation product or of other factors; it does seem, however, that only eukaryotic RNA's function in the oöcyte.

FLOW OF INFORMATION from nucleus to cytoplasm, the pathway leading to gene expression, might be subject to control at a number of points (*colored stars*). All genes are present in almost all cells, but different genes are expressed in different specialized cells: the genes for alpha globins and beta globins in red blood cells (*left*), for instance, and the gene for vitellogenin in liver cells of the female frog (*right*). The nucleotide sequences of the DNA (the genes) are transcribed into similar sequences of RNA. The initial transcript is probably into a precursor to which a string of adenylic acid nucleotides (*A*) is added; this premessenger RNA is processed to form messenger RNA, which leaves the nucleus and—if it is not masked or degraded—becomes attached to ribosomes and is translated into a polypeptide chain. The completed chain may be modified in various ways, as in the case of the proteins whose formation is illustrated. Two alpha and two beta globin chains are combined with one another and with four heme groups to form hemoglobin; the large vitellogenin chain is modified chemically and assembled into the yolk platelets in the oöcyte, where it is cleaved to form two yolk proteins.

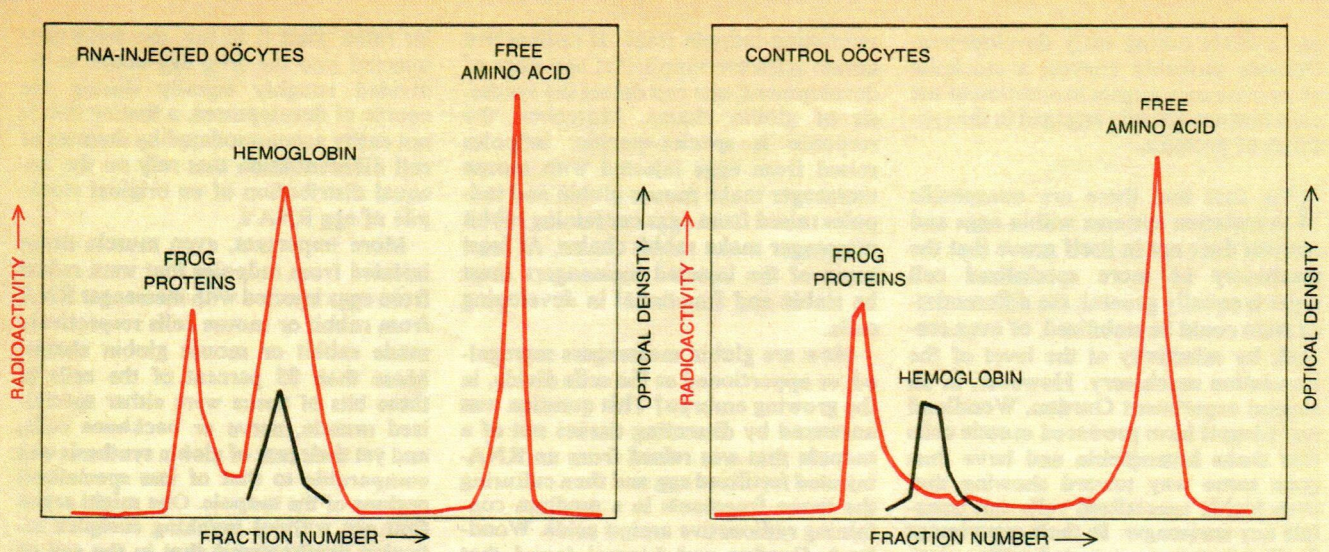


HEMOGLOBIN is synthesized in *Xenopus* oöcytes injected with messenger RNA from rabbit red blood cells. The messenger is extracted from reticulocytes, immature red cells, and is injected into oöcytes that have been removed from the ovary. The injected cells are incubated with a radioactive amino acid (*colored dots*), thus labeling the proteins that are synthesized. An extract of the cells, to which rabbit hemoglobin has been added as a marker, is passed through a Sephadex column. The column separates the various proteins according to size: radioactive frog-oöcyte proteins (*colored circles*), rabbit hemoglobin (*black disks*) and also a radioactive protein (*colored disks*) that subsequent analysis shows is hemoglobin (*see illustration on opposite page*).

For example, synthetic polymers consisting of repeated single nucleotides or repeating polynucleotide sequences do not seem to be translated. Neither do viral RNA's if they are from viruses that infect bacteria (the latter are prokaryotes and lack nuclei) rather than plants or animals.

The very general nature of this translation system suggests, if it does not prove, that any of the oöcyte's protein-manufacturing assemblies can be programmed by any eukaryotic messenger RNA. One can, of course, argue that cell type-specific messengers require specific translation factors, and that the frog cell contains a complete set of such factors. Since promelittin has never been found in any vertebrate tissue, however, the idea that a factor specific for promelittin messenger RNA preexists in the oöcyte seems farfetched. The oöcyte might possibly contain factors that are specific for classes of messenger RNA's, and promelittin messenger might happen to fall in one such class. The generality of translation by the oöcyte is most simply explained, however, by the lack of any requirement for translation factors specific to particular messenger RNA's. This is not to say that message-specific factors might not exist in more specialized cell types.

The oöcyte provides more than a set of nonspecific translation systems. The translation machinery is not isolated in a test tube but is part of the living cytoplasm and is influenced by it; the polypeptide chains formed under the direction of a foreign messenger are exposed to the enzyme systems present in the cytoplasm of the injected cell. When we programmed oöcytes with messenger RNA from lens cells of the calf eye, we were surprised to see not only that the lens protein crystallin was synthesized in the oöcytes but also that an acetyl group (CH_3CO) was added at the amino (NH_2) end of the protein chain. This is the same modification that crystallin undergoes when it is made in the calf lens cell itself. The secondary-modification reaction that results in the



OÖCYTE-SYNTHESIZED HEMOGLOBIN is identified by Sephadex chromatography. The various fractions, separated by size, are examined for radioactivity and for optical density, or color intensity. The charts show the results for oöcytes microinjected with rabbit messenger (*left*) and for control oöcytes that were injected with buffer solution only (*right*). In both cases a major peak in the optical-density curve (*black*) identifies the red rabbit hemoglobin added as a marker. Both radioactivity curves (*color*) have peaks for newly synthesized frog proteins and for unincorporated amino acid. The radioactivity curve for RNA-injected oöcytes, but not the one for control oöcytes, has another peak that coincides with the hemoglobin peak: the RNA-injected oöcytes appear to have synthesized hemoglobin.

acetylation of crystallin is not, in other words, confined to the highly differentiated lens cell. That some proteins are acetylated and others are not must stem from the specificity of the acetylating enzyme for particular amino acid sequences; provided only that those sequences appear (in this case on a polypeptide chain specified by a foreign RNA), the enzyme does its work. Secondary modifications of this type appear to be a consequence rather than a cause of cell differentiation. So far there has been no exception to the general finding that the secondary-modification reactions occurring in the specialized cells of vertebrates also occur in oöcytes, for example acetylation, hydroxylation, phosphorylation and polypeptide-chain cleavage. We expect to find certain exceptions, however. The inability of oöcytes to cleave insect promelittin into the secreted product melittin probably stems not from the invertebrate origin of the molecules but from a general lack in the oöcyte of the enzyme systems that are associated with external secretion.

The cytoplasmic milieu supports assembly processes as well as enzymic reactions. For example, the globin chains made under the direction of injected messenger RNA go on to combine with newly synthesized oöcyte heme; there is indirect evidence that some of the chains assemble, within the oöcyte, to form four-chain hemoglobin molecules. Injected messenger RNA for frog egg-yolk protein programs the synthesis of the giant polypeptide vitellogenin. The polypeptide is incorporated into yolk platelets and is cleaved by an enzyme into the main constituents of egg yolk, the proteins phosvitin and lipovitellin. Vitellogenin is normally made in the liver of the adult female frog and is then transported to the oöcyte; the experiment shows that the subcellular destination and processing of yolk proteins do not depend on the oöcyte's having absorbed vitellogenin from the bloodstream.

All this information narrows down the range of levels within which control must be mainly exerted. We have found that in the oöcyte the interaction between an available "free" messenger and the living cytoplasm leads automatically to translation, to secondary modification and even to assembly into multiple-chain proteins and into subcellular organelles. Other living cells may differ from the oöcyte in the precise nature of the controlling step, although many appear to be similar in that the flow of genetic information seems to be controlled at some level prior to the appearance of free messenger RNA; in the oöcyte, once the messenger is available the expression of genetic information follows as an automatic consequence. Qualitative regulation, then, implies the modulation of such mechanisms as transcription, the selection of particular molecules of premessenger RNA, the movement of messenger RNA out of the nucleus and the unmasking of stored messengers. The oöcyte and egg systems can be exploited for identifying and analyzing the critically important control elements that must operate at one of these pretranslation levels or more.

We found that the injection of either reticulocyte polyribosomes or complexes of messenger RNA with its attendant proteins leads, as the introduction of purified messenger RNA does, to the synthesis of globin chains. Tracing the possible control stages back toward the hemoglobin gene itself, the next logical question is whether or not the large messenger-RNA precursor has a globin-synthesizing capability. When Sau-Ping Kwan and I tested the globin-forming activity of precursor preparations derived from mouse and duck cells, the oöcytes did synthesize mouse and duck globins. The difficulty is that the oöcyte system detects minute amounts (less than a picogram, or a trillionth of a gram) of globin messenger, and so the precursor preparation must not be contaminated with even a trace of mature messenger. Contamination is hard to discern, because small messenger RNA's might aggregate to form what seems to be a large precursor molecule. We therefore mix in some rabbit-reticulocyte polyribosomes containing mature globin messenger; if what is intended to be a mouse or a duck messenger-precursor preparation makes any rabbit globin, it must be contaminated with mature messenger. Unfortunately that has been the case in all the experiments to date, and so we can only say it is probable that the information in messenger-RNA precursors can be translated by the oöcyte. The point remains to be proved.

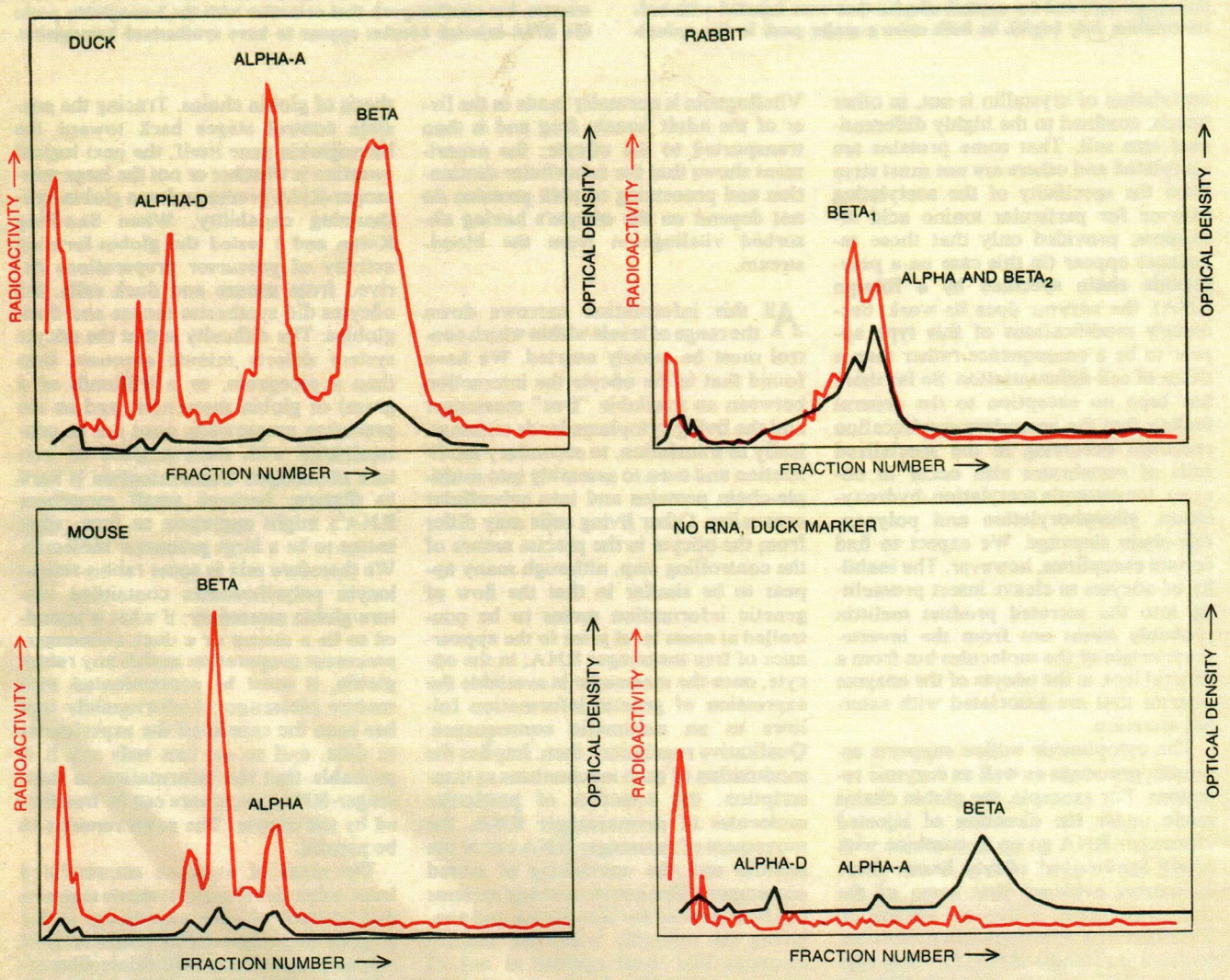
The mass of evidence accumulated from other developing systems suggests that control of gene expression at the level of messenger-RNA synthesis is of primary importance. Microinjection experiments, by showing the lack of qualitative control once messenger is available, also point to events occurring within the nucleus. The unmasking of preformed messengers, however, may also play an important regulatory role.

particularly during early development. Oöcytes probably contain a stockpile of inactive messengers in addition to the ones that are actively engaged in the synthesis of proteins.

The fact that there are nonspecific translation systems within eggs and oöcytes does not in itself prove that the machinery of more specialized cell types is equally general; the differentiated state could be stabilized, or even created, by selectivity at the level of the translation machinery. However, in an elegant experiment Gurdon, Woodland and Lingrel have produced muscle cells that make hemoglobin and have thus gone some way toward showing that even highly specialized cells can translate any messenger. In their experiment fertilized eggs are injected with mouse or rabbit globin messenger. Many of the eggs develop normally, right up to the swimming-tadpole stage. If radioactive amino acids are supplied at any stage of development, one can detect the synthesis of globin chains. Moreover, the response is species-specific: tadpoles raised from eggs injected with mouse messenger make mouse globin and tadpoles raised from eggs containing rabbit messenger make rabbit chains. At least some of the injected messengers must be stable and functional in developing cells.

How are globin messengers segregated, or apportioned as the cells divide, in the growing embryo? This question was answered by dissecting tissues out of a tadpole that was raised from an RNA-injected fertilized egg and then culturing the tissue fragments in a medium containing radioactive amino acids. Woodland, Gurdon and Lingrel found that several different tissues and regions of the tadpole made globin chains at similar rates. That is to say, the messenger injected into the frog egg seems to be divided roughly equally during the course of development, a finding that is not easily accommodated by theories of cell differentiation that rely on the unequal distribution of an original stockpile of egg RNA's.

More important, even muscle tissue isolated from tadpoles that were raised from eggs injected with messenger RNA from rabbit or mouse cells respectively made rabbit or mouse globin chains. More than 85 percent of the cells in these bits of tissue were either specialized muscle, nerve or backbone cells, and yet their rate of globin synthesis was comparable to that of less specialized regions of the tadpole. One might argue (but not without invoking complex selection mechanisms) that in the egg or during the formation of the embryo the globin RNA's had picked up specific



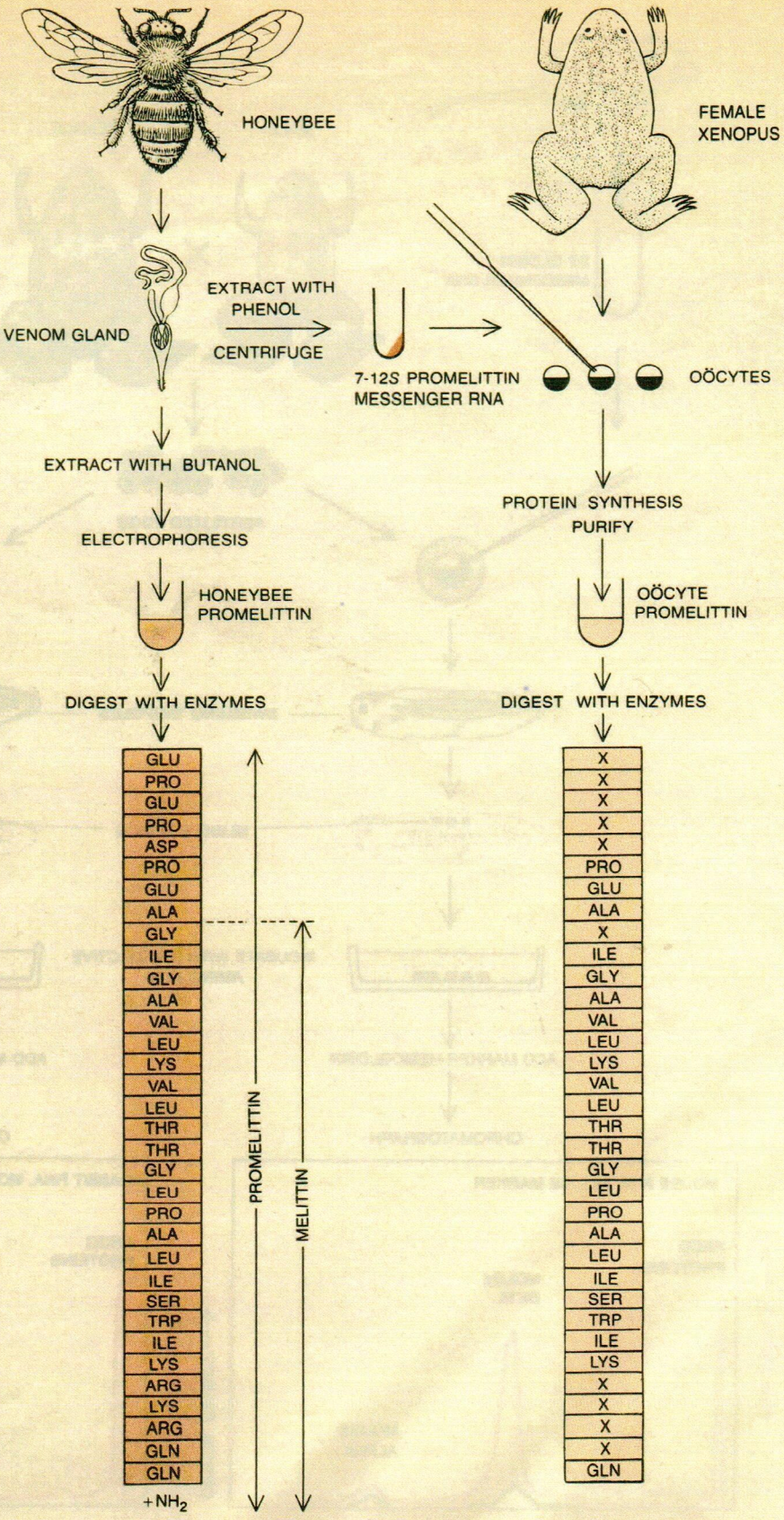
ABILITY OF OÖCYTE to translate the information encoded in a foreign RNA was established by repeating the experiment with RNA's from three different species and by breaking down the hemoglobin into globin chains, which can be identified by species. After injection with either duck, rabbit or mouse messenger RNA and incubation with radioactive amino acid, the oöcytes were mixed with the appropriate hemoglobin and an extract was made and passed down a Sephadex column. The partially purified hemoglobin thus obtained was subjected to ion-exchange chromatography. Radioactivity (*color*) and optical-density (*black*) curves showed that injected oöcytes produced globin chains that chromatographed with the appropriate marker chains; when no foreign RNA had been injected, there was no synthesis of globin chains (*chart at bottom right*). Note that ducks have two kinds of alpha chain, certain rabbits two kinds of beta chain.

factors that allowed them to be translated in the muscle cell. The simplest explanation, however, is that normal muscle cells are able to translate globin messenger if the globin RNA is available. It therefore seems likely that even specialized cells can translate any messenger. Taken in combination with the oöcyte and egg data, this result suggests that any cell can be programmed by any messenger.

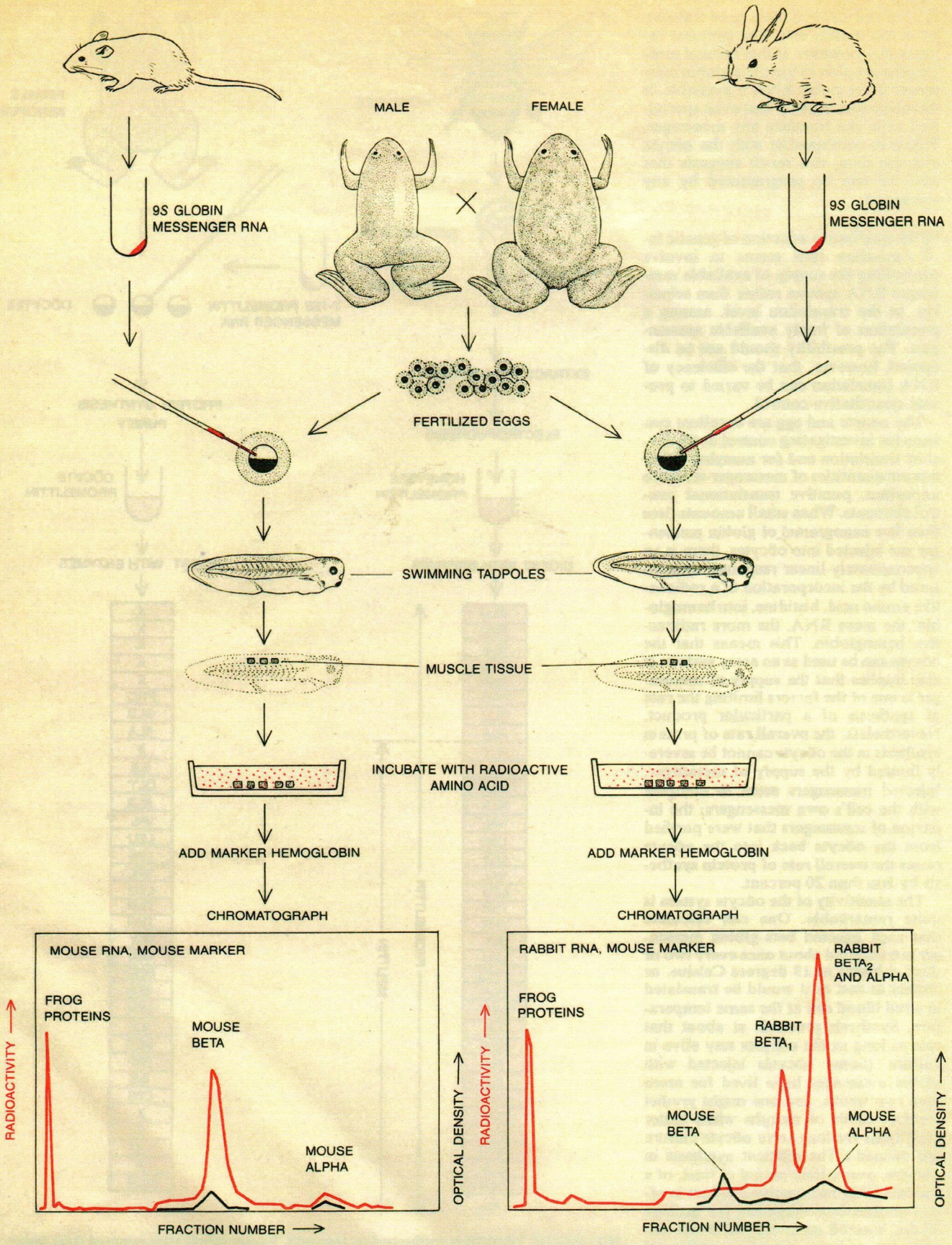
The qualitative selection of genetic information thus seems to involve controlling the supply of available messenger-RNA species rather than selecting, at the translation level, among a population of freely available messengers. The possibility should not be dismissed, however, that the efficiency of RNA translation can be varied to provide quantitative control.

The oöcyte and egg are excellent systems for investigating control at the level of translation and for assaying either minute quantities of messenger or, more important, putative translational control elements. When small amounts (less than five nanograms) of globin messenger are injected into oöcytes, there is an approximately linear response, as measured by the incorporation of a radioactive amino acid, histidine, into hemoglobin: the more RNA, the more radioactive hemoglobin. This means that the oöcyte can be used as an assay system. It also implies that the supply of messenger is one of the factors limiting the rate of synthesis of a particular product. Nevertheless, the overall rate of protein synthesis in the oöcyte cannot be severely limited by the supply of messenger. Injected messengers seem to compete with the cell's own messengers: the injection of messengers that were purified from the oöcyte back into the oöcyte raises the overall rate of protein synthesis by less than 20 percent.

The sensitivity of the oöcyte system is quite remarkable. One can calculate that each injected beta-globin messenger is translated about once every two or three minutes at 19 degrees Celsius, or almost as fast as it would be translated in a red blood cell at the same temperature. Synthesis proceeds at about that rate as long as the oöcytes stay alive in culture. (Some oöcytes injected with globin messenger have lived for more than two weeks, and one might predict survival times of months when better conditions for long-term oöcyte culture are defined.) The efficient synthesis in oöcytes, over a long period of time, of a stable, highly radioactive product in effect enormously magnifies the activity of the injected material and hence enables one to assay minute quantities of messenger—less than a trillionth of a gram of beta-globin messenger RNA. If, on the other hand, extreme sensitivity is not required, it can be more convenient to assay messenger RNA in a cell-



HONEYBEE VENOM is synthesized by frog cells. Venom glands were removed from honeybees and a messenger-RNA fraction was isolated. The messenger was injected into frog oöcytes, which synthesized promelittin, the precursor of the venom component melittin. The amino acid sequence of the oöcyte-synthesized promelittin (*right*) was compared with the sequence of the honeybee protein itself, removed directly from the venom gland (*left*). To the extent that it was determined, the sequence of the oöcyte promelittin was the same as the honeybee version. Secondary modifications such as addition of NH_2 were not carried out in oöcyte.



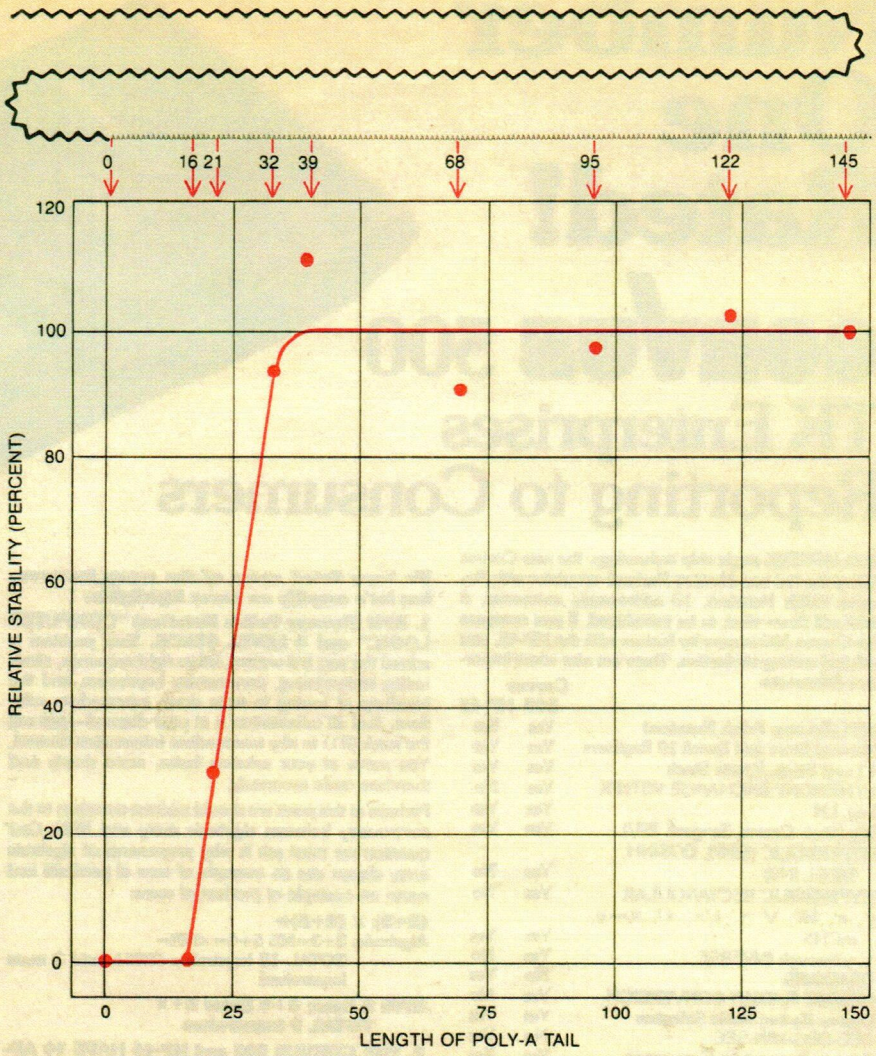
SPECIALIZED CELLS can also translate foreign messenger. At the Laboratory of Molecular Biology in Cambridge, Hugh R. Woodland, John B. Gurdon and Jerry B. Lingrel raised tadpoles from normally fertilized eggs into which messenger RNA for mouse or rabbit globin had been injected. Muscle tissue was dissected from tadpoles and incubated with a radioactive amino acid; mouse hemoglobin was added as a marker. Ion-exchange chromatography shows that chains synthesized in the tissue correspond to the species of the injected messenger: chains made under the direction of mouse RNA form radioactive peaks that coincide with mouse marker peaks (*left*); chains programmed by rabbit messenger yield peaks characteristic of rabbit globin and hence not coincident with the mouse marker peaks (*right*).

free system, where the confusing background of frog-protein products is not present. For assaying putative translational control elements, however, the oöcyte is best because what happens in it is likely to be relevant to what happens in normal living cells. For such purposes one can mix possible regulatory molecules with a messenger RNA and inject the combination or else inject the messenger and, once it is established, introduce the regulator.

The approach is illustrated by an experiment to determine the role of heme in controlling the translation of globin messenger. Crude globin messenger contains just a little more alpha messenger RNA than beta messenger RNA and programs the synthesis of roughly equal amounts of the two chains in a cell-free system. In contrast, oöcytes injected with mouse or rabbit messenger make at least five times as much beta globin as alpha globin. If heme is mixed with the messenger or is injected after the messenger is established, the translation of alpha messenger is enhanced so that the alpha/beta ratio approaches unity. This effect does not necessarily imply that there are elements of the translation system that interact with only one messenger. Rather, the heme probably increases the supply of factors that initiate polypeptide synthesis and thus preferentially affects the alpha messenger, which has a lower affinity for these initiation factors than the beta messenger.

The fertilized egg is also a good medium for studying translational control elements. Components injected into a fertilized egg end up in the rapidly dividing and differentiating—and hence biologically interesting—cells of the growing embryo. In one experiment of this kind the injected messenger served as a probe for the appearance of particular factors during the course of early development. We introduced mouse or rabbit globin messenger and measured the alpha/beta ratio at various developmental stages. Mouse messenger yields the characteristic excess of beta chains. So does rabbit messenger, in the egg and during the initial cleavage stages, but just before the stage called gastrulation the synthesis of alpha chains increases, and it remains at an elevated level at least until the swimming-tadpole stage [*see illustration on page 71*]. Some factor, similar to heme in effect, that specifically stimulates the translation of rabbit alpha-chain messenger appears to be formed at a particular developmental stage.

Whole-cell assay systems present an excellent opportunity for studying messenger stability. The lifetime of a messenger in a cell-free system sheds little light on what its life expectancy would be in a normal living cell; yet to study a cell in which the messenger is being synthesized is difficult, since at any given



FUNCTIONAL STABILITY in oöcytes of globin messenger molecules with poly-A tails of different lengths was tested by a Belgian-Israeli group. By treating globin messenger RNA with the enzyme polynucleotide phosphorylase for varying periods they produced messenger species with tails of different lengths; the species were purified by chromatography and then injected into oöcytes. After 44 hours of incubation a radioactive amino acid was added and the rate of hemoglobin synthesis was compared with the rate in oöcytes programmed with native, or unshortened, globin messenger. Some 30 adenylic acids are required to stabilize messenger.

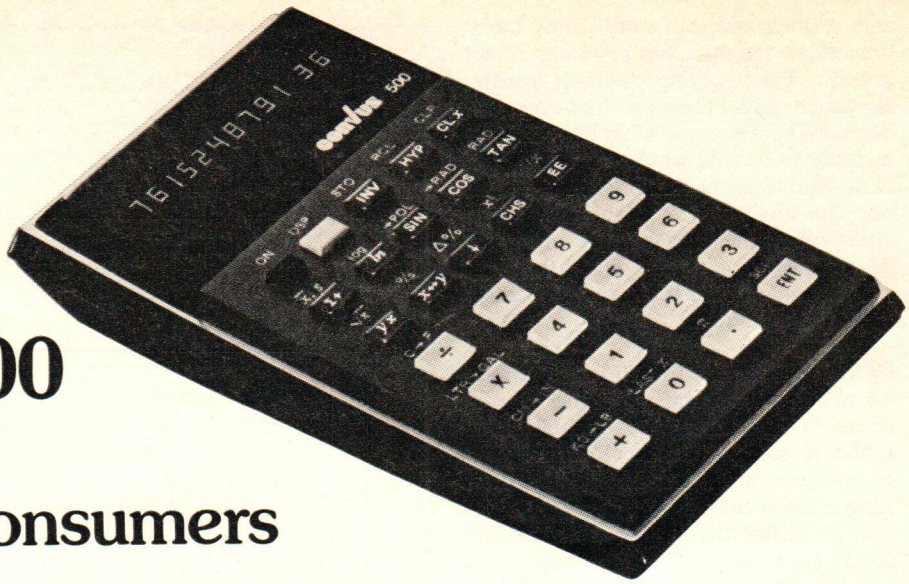
time the messenger population reflects a balance between synthesis and decay. By injecting a known messenger into an egg or an oöcyte one can study the decay process alone. Messenger stability is relevant to the control of gene expression because instability could be a means of changing the population of proteins as cells differentiate. In this regard results obtained with fertilized eggs are likely to be of particular interest, since here one can look at messenger stability in a developing embryo. So far only rabbit and mouse globin messengers have been tested in this way, and they both appear to be stable at least until the swimming-tadpole stage, after more than 20 cell divisions. Rabbit globin is also stable in the oöcyte, and injected oöcytes will synthesize globin for several weeks. Two frog-liver messengers, those coding for albumin and for the enormous vitellogenin molecule, are also stable, showing that stability is not an artifact arising from the introduction of foreign messenger. (All the messengers so far tested code for differentiated products and are stable in their normal cellular milieu; messengers with a short half-life have not been injected into eggs or oöcytes, and it is possible that they would retain the characteristics expressed in the cell type from which they were derived.)

Perhaps the most elegant exploitation of the oöcyte system for investigating messenger-RNA stability has been the work of a group of investigators from the Free University of Brussels (G. Huez, Marbaix, E. Hubert and M. Leclercq) and the Weizmann Institute of Science in Israel (Uri Nudel, Hermona Soreq, R. Salomon, B. Lebleu, Michel Revel and Uriel Z. Littauer), who studied the part played by the "poly-A tail" of the messenger RNA in determining

Number One Rated!

corvus 500

TK Enterprises Reporting to Consumers



With MOSTEK single chip technology, the new Corvus 500 is the first non-Hewlett-Packard calculator with Reverse Polish Notation, 10 addressable memories, 4 level roll down stack to be introduced. If you compare the Corvus 500 feature by feature with the HP-45, you will find striking similarities. There are also some important differences:

	Corvus 500	HP-45
RPN (Reverse Polish Notation)	Yes	Yes
Memory Store and Recall 10 Registers	Yes	Yes
4 Level Stack, Rotate Stack	Yes	Yes
10 MEMORY EXCHANGE WITH X	Yes	No
Log, LN	Yes	Yes
Trig (Sine, Cosine, Tangent, INV)	Yes	Yes
HYPERBOLIC (SINH, COSINH, TANH, INV)	Yes	No
HYPERBOLIC RECTANGULAR	Yes	No
y^x, e^x, 10^x, $\sqrt{}$ ×, 1/×, ×!, ×↔y, π,CHS	Yes	Yes
$\sqrt[x]{y}$ through INVERSE	Yes	No
GRADIANS	No	Yes
DEGREE-RADIAN CONVERSION	Yes	No
Degree-Radian Mode Selection	Yes	Yes
DEC-DEG-MIN-SEC	No	Yes
Polar to Rectangular Conversion	Yes	Yes
Recall Last ×	Yes	Yes
Scientific Notation, Fixed and Floating	Yes	Yes
Fixed Decimal Point Option (0-9)	Yes	Yes
DIGIT ACCURACY	12	10
DISPLAY OF DIGITS	12	10
%, Δ %	Yes	Yes
GROSS PROFIT MARGIN %	Yes	No
Mean and Standard Deviation	Yes	Yes
Σ+, Σ−	Yes	Yes
Product—Memories	Yes	Yes
C.F. DIRECT CONVERSION	Yes	No
F.C. DIRECT CONVERSION	Yes	No
LIT-GAL, DIRECT CONVERSION	Yes	No
KIL-LBS, DIRECT CONVERSION	Yes	No
GAL-LIT, DIRECT CONVERSION	Yes	No
LBS-KIL, DIRECT CONVERSION	Yes	No
CM-INCH DIRECT CONVERSION	Yes	No
INCH-CM DIRECT CONVERSION	Yes	No

As you can see, the Corvus 500 IS a lot more calculator for $69.95.

We have listed some of the many features, but let's amplify on some highlights:

1. RPN (Reverse Polish Notation) "COMPUTER LOGIC" and 4 LEVEL STACK. Your problem is solved the way it is written, left to right sequence, eliminating restructuring, unnecessary keystrokes, and the handicap of having to write down intermediate solutions. And all information is at your disposal—just roll the stack (R↓) to any intermediate information desired. You arrive at your solution faster, more simply and therefore more accurately.

Perhaps at this point we should address ourselves to the controversy between algebraic entry and RPN. One question we must ask is why proponents of algebraic entry *always* use an example of sum of products and *never* an example of product of sums:

(2+3) × (4+5)=

Algebraic: 2+3=MS 5+4=×MR=
TOTAL 12 keystrokes (SR51, add 2 more keystrokes)

RPN: 2 Enter 3+4 Enter 5+×
TOTAL 9 keystrokes

2. THE CORVUS 500 and HP-45 HAVE 10 ADDRESSABLE MEMORY REGISTERS, 4 LEVEL OPERATIONAL STACK, and a "LAST X" REGISTER (10th Mem. Reg.) With 10 addressable memories, you have access to more entries, or intermediate solutions; less remembering, or writing down, YOU have to do. And less chance for error.

The stack design also permits X and Y register exchange, and roll-down to any entry to the display for review or other operation.

The "last x" register permits error correction or multiple operations when a function is performed, the last input argument of the calculation is automatically stored in the "last x" register, which can be quickly recalled to correct an error, or to perform another operation using the same number.

3. DIRECT HYPERBOLIC and HYPERBOLIC RECTANGULAR to POLAR, and INVERSE. For those of you electronic and computer science engineers who require access to this specialized application, the Corvus 500 solves "your" problems.

4. A WORD ABOUT CORVUS 500 12 DIGIT DISPLAY AND ACCURACY. Finally you have displayed 12 digit accuracy in business format and 10 + 12 in scientific notation. LED is manufactured by Hewlett-Packard.

To order your Corvus 500 Scientific Calculator, phone:* (800) 421-0367; (213) 370-5795 *or mail coupon today!

FOR THE FIRST TIME you can raise the number 10 to 199th power or calculate Factorial (x!) of up to 120. Unbelievable!

5. The CORVUS 500 CONVERTS METRIC/U.S. and INVERSE UNITS DIRECTLY. These preprogrammed functions act not just as constants, eliminating unnecessary keystrokes.

WHAT ABOUT CONSTRUCTION? With so many features, the next most obvious question must be in regard to the quality of the unit itself. We are proud to report the Corvus 500 to be double injected molded, with "tactile" feedback keyboard. The compact, contoured case is 5½" long by 3" wide by 1¼" high, and weighs just 8 oz.

The COMPLETE CORVUS 500 for $69.95 includes:

- **Rechargeable and replaceable Nickel Cadmium batteries. Optional 3 AA batteries.**
- **Adaptor/Charger**
- **Owner's handbook**
- **Soft carrying case**

The Corvus 500 is warranted by the manufacturer against defects in materials and workmanship for one year from date of delivery.

For those of you who have the HP-21 or 45 or any other advanced calculator on order, aren't you glad you still have the opportunity to take advantage of the release of the Corvus 500 for $69.95? Hurry! Order yours today.

***AN INVITATION:** TK Enterprises is proud that we are able to introduce the MOSTEK chip Corvus-500 to you, our discerning readers. (Incidentally, we have received orders for Corvus 500 from almost every major company in the USA, in every state in the Union (including HP & TI people). Many are already taking advantage of this incomparable price performance ratio instrument. We invite you to experience the new Corvus 500 by mailing in the coupon today, or call our order desk* **(800) 421-0367; in CA. (213) 370-5795.** *From the tremendous initial response, please allow 3 days for delivery. We are sure you will agree that the CORVUS 500 is the calculator of the year, and the one you have been waiting for. However, you may return the remarkable Corvus 500 within 7 days without obligation, for any reason whatsoever. So, please mail or phone today . . . before the news of Corvus 500's demand exceeds supply!*

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Yes . . . I'd like to try the Corvus 500 for 7 days. Please ship me the Corvus 500 Advanced Scientific Calculator, complete with accessories, at $69.95, plus $2.50 for shipping and insurance. I understand that if I'm not completely satisfied, I may return the unit and accessories within 7 days for full refund (or, at my option, receive full credit toward the purchase of any other calculator carried by T.K. Enterprises).

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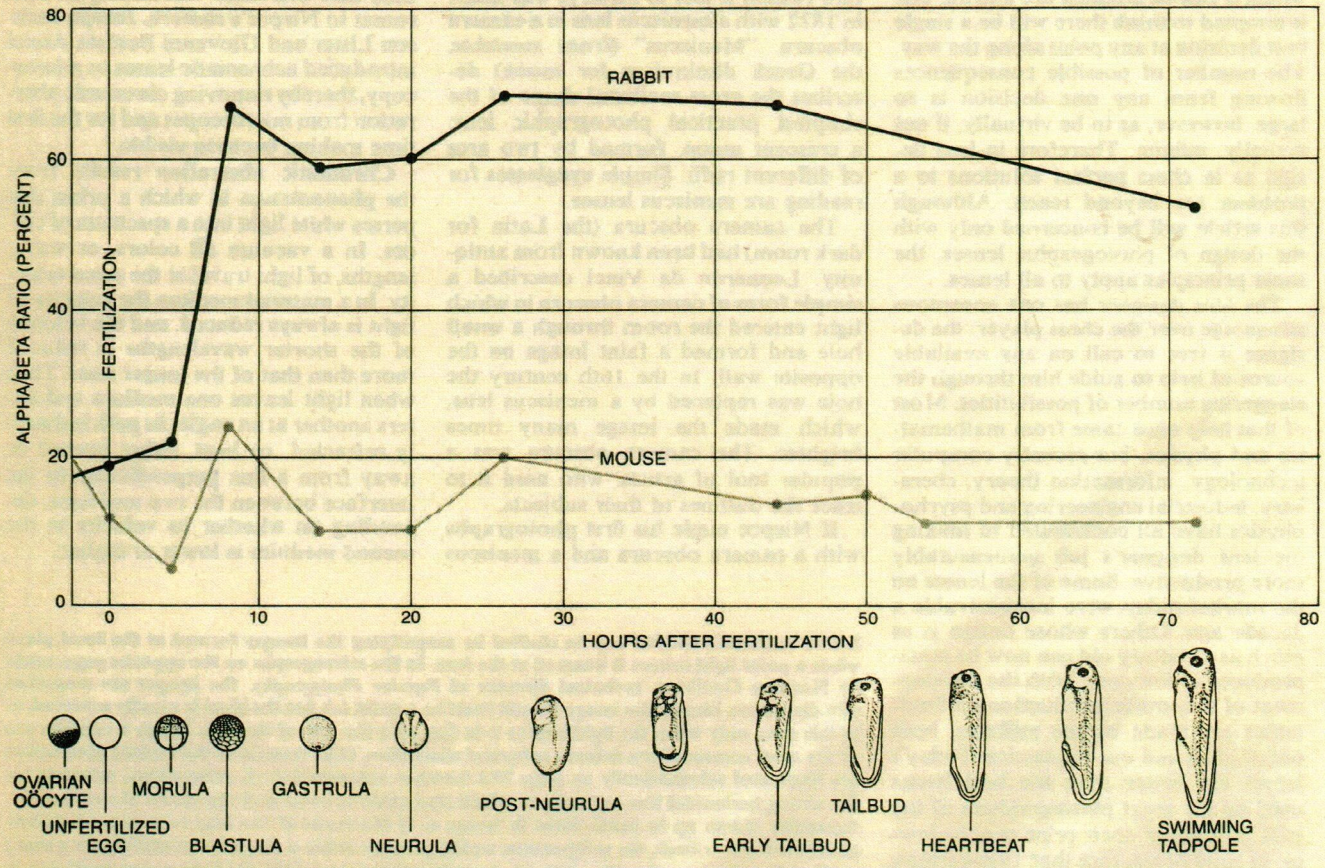
the life expectancy of the whole molecule. One of the most distinctive features of most kinds of messenger RNA is the presence at one end of the molecule of a long string of a single kind of nucleotide, adenylic acid. Yet the function of these rather striking poly-A tails remained a mystery, not least because it was known that messengers lacking tails could be translated normally in certain cell-free systems. The Belgian-Israeli group injected into oöcytes rabbit globin messenger RNA with and without poly-A tails. They found that messengers without tails are translated well enough at first but do not function efficiently for very long: after 48 hours the RNA with poly-A was making about 25 times as much globin as the tailless RNA. The poly-A is thus implicated in preserving the functional stability of messenger RNA. The tailless messengers are actually destroyed by the oöcyte; they can hardly be detected physically 48 hours after injection. Since the tails were removed by stepwise degradation with the enzyme polynucleotide phosphorylase, one might argue that the enzyme also affected some other part of the globin messenger, for example the region adjacent to the tail. The investigators went on, however, to restore poly-A tails to the RNA with the enzyme adenyl transferase, and they found that stability was restored. In other words, it really is the presence of the tail that confers stability on the messenger molecule.

When polynucleotide phosphorylase is used to shorten the tail rather than to remove it completely, a critical tail length of about 30 adenylic acid units turns out to be required before a messenger molecule is stabilized [*see illustration on page 69*]. One can speculate that in cells that, unlike the oöcyte, require a rapidly changing population of messengers the life expectancy of a "stabilized" molecule may depend on how much longer the tail is than the critical length, given that many such cells contain enzymes that shorten poly-A tails. Although many of the above results could be peculiar to the oöcyte system, careful reexamination of data from cell-free systems has confirmed the tendency of messenger without poly-A to be less stable. With their combination of sophisticated chemistry and simple microinjection experiments the Belgian-Israeli group may well have solved the mystery of the poly-A tail.

Experiments in the microinjection of RNA demonstrate the advantages of combining the unrelated disciplines of biochemical analysis and micromanipulation. The approach is not limited to amphibian eggs and oöcytes. The egg of the fruit fly *Drosophila* is several hundred times smaller than the *Xenopus* oöcyte and the mouse egg is several thousand times smaller, yet both of these small eggs can be microinjected. Ordinary cells growing in culture have also been injected, and the technique is not limited to the injection of RNA. Gurdon, Alan Colman, Christopher Ford and Woodland have already injected eggs and oöcytes with eukaryotic and viral DNA and with histones, antibodies and other proteins. Some synthetic and naturally occurring DNA's are transcribed, and the DNA replicates in the egg cytoplasm. Introducing specific genes into living frog cells could improve our understanding of development. The raw materials for such experiments might be provided by recently developed techniques for cloning recombinant DNA molecules.



CONTROL OF TRANSLATION at various developmental stages is studied by injecting mouse or rabbit globin messenger into fertilized eggs and analyzing the globin synthesized by the embryos for its content of alpha and beta chains. Eggs injected with mouse RNA produce about a fivefold excess of beta chains (*gray curve*), that is, the alpha/beta ratio is about 20 percent (as it is also in injected oöcytes). In eggs programmed with rabbit globin messenger the alpha/beta ratio is about the same at first (*black curve*), but production of alpha chains increases suddenly just before gastrulation; some factor that stimulates alpha-chain synthesis is formed at that stage of development.