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Theoretical Biochemical Constructs Related to Normal and Abnormal Palatal Embryogenesis

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The pathogenesis of the formation of the cleft palate was initially focused on theories evolving from the anatomy of the palate. The classical theory separates the development of the central third of the face and the premaxilla from the development of the hard and soft palate (*27*). This concept envisages various peninsular masses of ectoderm and mesoderm which are surrounded by a free space or a cleft. These peninsular masses, called processes, are thought to grow, to meet, and to fuse in much the same manner as the healing of the wound. It is maintained that the local inhibition of normal development would prevent normal fusion of the facial processes, resulting in a cleft. The differentiation of the areas involving the central portion of the face may in itself be labile to physiological and physical stresses. In addition to this, it is possible that the control of specialization of the cleft region may be mediated by processes well known in other areas of embryologic development (*9, 13, 16, 17, 24, 28*).

A vast variety of substances and events may lead to the formation of a cleft palate (*7, 13, 23*). The synthesis of collagen and the sulfation of acid mucopolysaccharides are easily inhibited by cortisone-like drugs at reasonably low levels. At higher levels these hormones decrease cell division. Avitaminosis decreases synthesis of protein, enzymes, and ribosomal activities. Lack of adequate protein nutrition may lead to decreased synthesis and interference with the timing of events in differentiation. Physical trauma either arising from the three dimensional stresses of growth at a time when fusion is about to take place or following the low strength of the epithelial plates may lead to tearing

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of the fusion sites or a widening of the profusion area. Similarly, external trauma to the womb at any time before the fusion site has developed strength could account for the cleft palate formation.

The differentiation of specialized tissues in the process of embryological development is an area of concern to a wide variety of scientific specialties. Within the past twenty years, the field of biochemistry of genetics has progressed to a point where many phenomena previously obscure may be explained in part or in total by events that take place at the molecular level. It is well known that organizer nodes are developed at specific time intervals in the differentiation of embryo tissue. These include limb buds, organ buds, et cetera (*26*). The organizer nodes concerned with the development of undifferentiated tissue to the differentiated state contain diffusible substances which may be used experimentally to study this process. Studies by Niu, Twitty, and others have shown that saline extracts of organizer nodes, when incubated with presumptive ectodermal tissue, can cause the selective differentiation of such tissue in a direction similar to that of the node extract utilized (*19, 20, 21*). Extracts derived from the organizer node for kidney tissue cause the differentiation of presumably ectodermal tissue from the blastula stage into tissue somewhat similar in organization and structure to that of kidney. Similarly, it has been shown that extractable materials from the organizer node of the thymus leads to the development of undifferentiated primitive ectoderm into thymus-like architecture. A wide variety of other evidences of the extracts from organizer nodes on undifferentiated tissues have been reported. The presence of RNA in the organizer node extracts was evidenced by the presence of a typical absorption spectra in the ultraviolet region. Furthermore, selective extraction technics, which allow only RNA to be extracted, led to the same directed types of differentiation. In order to understand the nature of differentiation as related to the cleft palate is an error in genetically dictated differentiation or in the relationship of the diffusible substances arising from a node which organizes and directs such development. The process of fusion of the epithelial rods between the 45th and 54th day of development of the human fetus undoubtedly is an event of regional differentiation (*14, 31*). Questions that may be asked are: What are the known biochemical changes? What embryological changes are involved in the fusion of the two epithelial rods into a single plate? Does this involve the synthesis of specific enzymes which alter the adhesiveness of the cells in the region of fusion, the release of a limited level of a proteolytic or polysaccharide synthesizing enzyme to render the cells partially adherent or sticky? Or does this involve a complex of many changes that are controlled by the local genetic pattern? In order to discuss the areas of theoretical biochemical possibilities, it is necessary to outline the known molecular events that are involved in the normal sequence of DNA-RNA protein

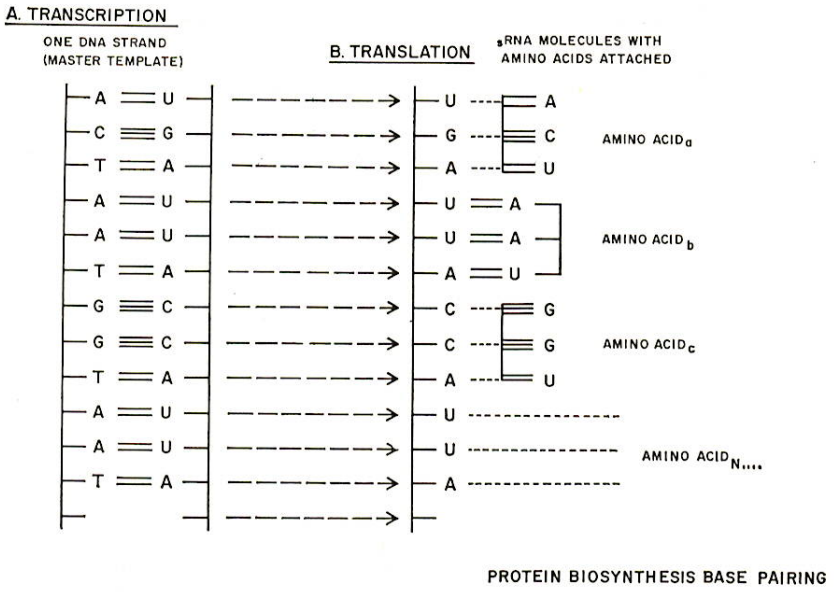
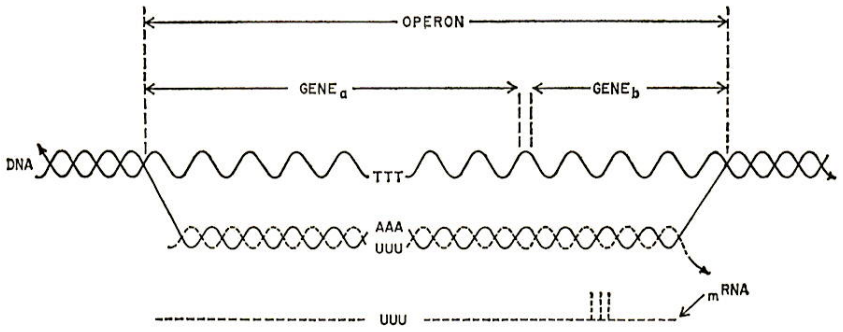


FIGURE 1. *Transcription and Translation.* The DNA master template permits the specific translation from one strand of DNA to mRNA. The subsequent translation from mRNA to the specific coded amino acids a, b, c . . . n during protein synthesis are depicted.

biosynthesis (structure and enzyme formation). The concept that nucleic acids may be involved has previously been verbally presented by one of the authors (DBC) at an annual seminar of the Lancaster Cleft Palate Clinic.

The Operation of Genes

Cytological development is dependent upon a complex variety of biochemical events which are controlled by genes. The genetic material (genes) is composed of high molecular weight deoxyribonucleic acid (DNA). The four structural units within DNA which control the genetic code are adenine, thymine, guanine, and cytosine (*2*). These bases represent the four letters of the genetic code and may be abbreviated A, T, G, and C (Figure 1). These four letters thus provide all of the code information for the incorporation of twenty different amino acids and specify precisely the proper sequence in polypeptide chains and in proteins of each amino acid (Figure 2). This DNA exists in the form of a doubly stranded right-handed helix (*30*). The specific pairing of the bases within the helix lends stability to the structure. Adenine pairs are extremely important in the replication of DNA. Prior to cell division, it is essential that each cell provides a doubly stranded helix of DNA composing all of the information present within the original double helix (*11, 12*). The DNA master strand thus is



GENETIC CONTROL OF PROTEIN BIOSYNTHESIS

FIGURE 2. *Genetic Control of Protein Biosynthesis.* An operon is a linear array of genes whose structural activity is controlled by an operator gene (a functional gene) located on one end of a DNA strand. The range of the operon illustrated includes genes a and b. The operon represents the length of the DNA molecule (in this case, genes a and b) whose complementary strand of RNA comprises a single intact segment of mRNA. The operon, therefore, controls one unit of mRNA transcription. A portion of gene a illustrates coding. One DNA triplet of TTT on one strand of DNA is specifically paired with AAA. This triplet thus codes for UUU in the mRNA strand.

capable of replicating to form new DNA of the same composition for the daughter cell. This is mediated by virtue of the specific base-pairing sequence. The transfer of genetic information from the nucleus to the cytoplasm of the cell is accomplished through a high molecular weight polymer which is termed ribonucleic acid, RNA. This nucleic acid resembles DNA but differs from DNA in containing thymine in place of uracil and ribose in place of deoxyribose. Unlike DNA, RNA exists as a single strand and does not assume a totally helical configuration. The guanine pairs with cytosine in the two strands of DNA in the double helix and thus guanine in a DNA strand may pair with a cytosine of a ribonucleic acid; the reverse is also true (*10*). The adenine of DNA pairs with the uracil in RNA. The thymine in DNA will pair with the adenine in RNA. Thus a mechanism exists for the specific base pairing and the transfer of the code information from DNA to RNA (Figure 2). A single strand of RNA can form a complementary pair (hybrid) with a strand of DNA (*8*). Base pairing provides a system by which the sequence of bases in a nucleic acid chain may be transferred from DNA to RNA. In protein biosynthesis, DNA serves as the primary template for a messenger RNA (mRNA) which complements the DNA base sequence. This messenger RNA thus can serve as a template upon which sRNA molecules may be oriented. The sRNA molecules are of lower molecular weight and serve for the transfer of specific amino acids (*12, 15*). The amino acids are linked by peptide bonds to form a polypeptide chain. The sequence of this chain has thus been dictated by the original DNA code and further

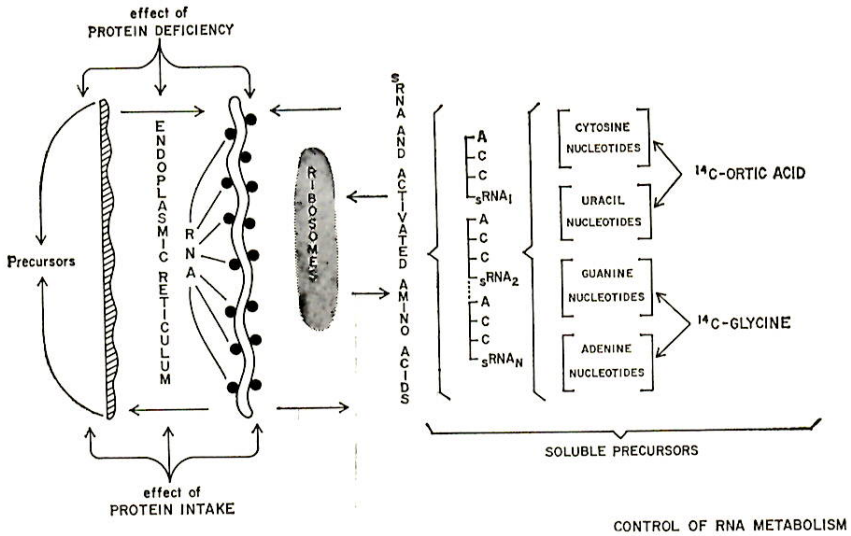


FIGURE 3. *Control of RNA Metabolism.* Messenger RNA (mRNA) residing in the crypts of rough endoplasmic reticulum or within nuclear ribosomes combine with soluble RNA (sRNA) and activated amino acids. The availability of the soluble precursors (sRNA and activated amino acids) at the level of the nuclear ribosome or the endoplasmic reticulum control synthesis of protein and RNA. Rates of synthesis may be followed experimentally via the use of radioactivity tagged soluble precursors.

carried through by the messenger RNA and finally transferred and translated by the sRNA through the mechanisms provided by the base-pairing rules (Figure 3). The first step in protein synthesis from the RNA template involves the reaction of an amino acid with ATP which is catalyzed by a specific amino acyl RNA synthetase (*3*). The product of this reaction is the enzyme-bound amino acid adenylate. This structure reacts with sRNA and forms the sRNA amino acid compound (Figure 4). Not only does each activating enzyme have a high affinity for ATP and for a specific amino acid, but it also exhibits a high degree of specificity for a particular species of sRNA molecule. These sRNA molecules are synthesized in particular regions of the genetic material and each species of sRNA apparently reflects a complementarity in base composition to its particular gene in the DNA. Each molecule of sRNA contains a unique short sequence of nucleotides which allows it to bind specifically to the proper complementary series of bases on the mRNA template (*18, 25, 29*). These events take place at sites within the membranes of the cytoplasm called the endoplasmic reticulum. The structures which contain the RNA on the endoplasmic reticulum are termed ribosomes (Figure 3). Ribosomes differ in size and base content from sRNA. The ribosomes active in protein synthesis are aggregated into multiples presumably linked by mRNA into polysomes. These polysomes are the workbenches through which sRNA molecules

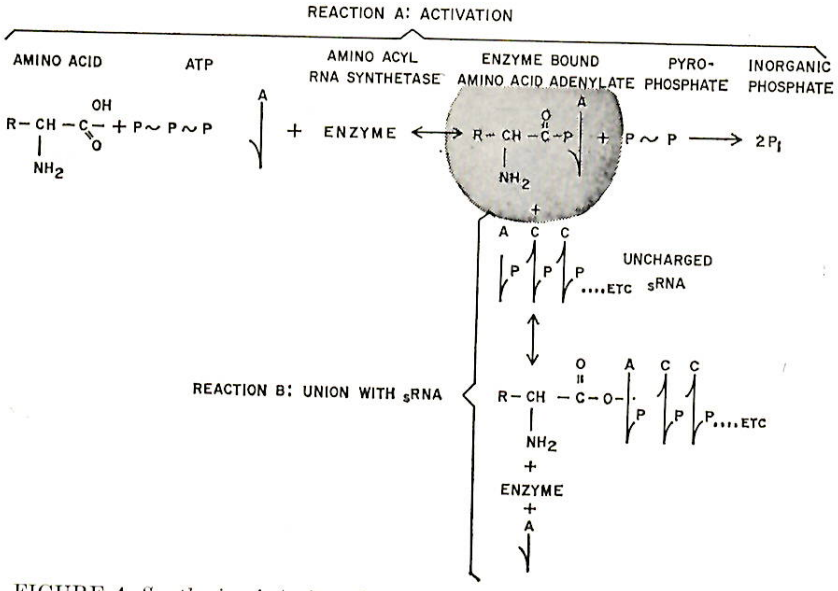


FIGURE 4. *Synthesis of Activated Amino Acid and Union with sRNA.* Amino acids and ATP in the presence of amino acyl synthetase react to form enzyme bound amino acyl adenylate plus pyrophosphate. The enzyme complex reacts with unchanged sRNA to form amino acyl sRNA.

can shuttle, transferring amino acids for the synthesis of polypeptide chains. The specific order in which these amino acid charged sRNA molecules are utilized depends upon the code contained in the mRNA molecules bound to the ribosomes. The overall pattern of the primary information transfer from DNA and its ultimate expression at the level of specific protein synthesis is shown in Figure 3.

The Mechanics of Gene Expression

The sequence of events between the primary information embodied within DNA and its ultimate expression in the protein synthesized is complex. All cells contain the same number of chromosomes and thus all are apparently capable of producing every type of molecule present within the body. This we know is not the case since the synthesis of insulin takes place only within highly specialized cells of the pancreas, the synthesis of growth hormone takes place specifically within the pituitary, and the synthesis of proteolytic enzymes takes place within the pancreas and liver. What then is the control mechanism which determines within a particular cell the spectrum of molecules that it is capable of synthesizing? Within recent years, it has been shown that DNA is covered by basically charged polypeptides—histones (*1, 4, 6*). These basically charged polypeptides which specifically coat certain regions of the DNA are selectively removed from the DNA strand and the molecule of RNA is synthesized complementary to the

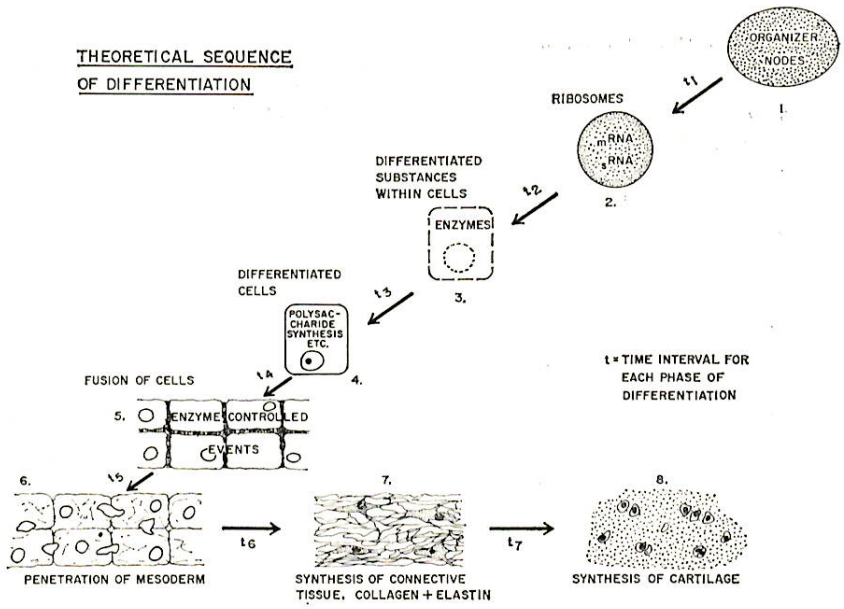


FIGURE 5. *Theoretical Sequence of Differentiation.* The organizer nodes (1) at time interval (t_1) elaborate ribosomes (2) which may in turn synthesize a de-repressor or activator polypeptide during t_2 which causes the specific differentiation of undifferentiated cell (3) which during t_3 synthesized the substances elaborated in differentiation (polysaccharides, enzymes, etc.) which during t_4 results in the fusion of cells which also are enzymic and time controlled events in differentiation. During t_5 the postulated necessary penetration of mesoderm (6) by cells which synthesize connective tissue during t_6 and finally the synthesis of cartilage (8) during t_7.

DNA portion (5). This process is highly specific: only the regions within the DNA strand which are specified in the differentiation of the particular cell are allowed to become replicated in the form of RNA and it is possible at this level that the specific differentiation of cells exists. For example, the portion of the DNA molecule concerned with the synthesis of a particular muscle protein may not be exposed in an epidermal cell. Similarly, the specific elaboration of hormones, enzymes, specific structural proteins, and all molecules concerned with the process of differentiated tissues may be specifically controlled. In the case of the cleft palate, we are drawn to the interesting sequence of events which occurs in the period from the 45th through the 54th day of human embryo development (*14*). On or about the 52nd day, the fusion of the two epithelial rods leading to the closure of the palate from the anterior to the posterior takes place. The various possibilities of control of the differentiation of the palate in fusion and further development are shown in Figure 5. Is this fusion mediated by the specific differentiation of the cells in the region to produce a sticky, gummy matrix which causes the adherence of the epithelial plates, by the release of a proteolytic enzyme which alters the electrical charges

on the surfaces of the cells and thus facilitates their adhering to each other, or is the change a more subtle one involving the synthesis of specific proteins and/or polysaccharides or other molecules? At this stage, we cannot specify which of these various possibilities are the likely ones. It is tempting however to think in terms of control mechanisms similar in nature to control mechanisms involved in the directive specialization of other tissues in embryogenesis (Figure 5). One must also consider the timing of specialization events. It is possible that the elaboration of the substance or substances required for the fusion of the epithelial rods may occur too early and thus not be available at a point in time when the cellular structures are suitable for the other embryological changes to take place or the event may happen at a later point in time too late to assist the fusion mechanism (Figure 5, $t_{1\text{-}4}$). This is, of course, hypothetical and no answers are possible at this point. Extracts of the epithelial rods prepared prior to the 52nd day, when incubated in the presence of the fusing site of a cleft palate animal, may provide an experimental tool for determining whether this developmental defect is indeed due to a lack of soluble, diffusible, differentiation factors.

A separate but distinct possibility may involve a mutational event which does not express itself through differentiation. In this case, the mutational defect may be expressed through means of a defective, genetic coding for the synthesis of the specific differentiator. In other words, there could be a misspelled code, an error in the transcription of the DNA code, or an error in the sequence of the DNA code. Such a mutation may also be evidenced through a defective expression device, that is, the basic peptide which controls the covering or uncovering of the active DNA site may be interfered with.

In order for normal differentiation to proceed beyond the fusion of the epithelial plate it is essential that mesodermal cells invade the epithelial wall that will later form the premaxilla and upper lip (Figure 5, phase 6). Synthesis of collagen, elastic fiber and mucopolysaccharide within structural support occur (Figure 5, phases 7 and 8). The activation or release of these differentiators would require precise timing since invasion by mesodermal cells may require a complex pattern of differentiation. We do not know whether some form of organizer is needed to activate this process. What, if any, are the enzymes required by the mesodermal cells to achieve penetration across this wall (Figure 5, $t_{2\text{-}4}$)? Are proteolytic and/or polysaccharide hydrolyzing enzymes released to accomplish this penetration? These areas of ignorance will be illuminated by biochemical investigation.

Perspectives

Our concepts in the past of the nature of hereditary material, its role in the differentiation of tissues, and its final expression in the

development of the organism have continually undergone revision. As is the practice in science, it is necessary that our present concepts be tested and changed. As more experimental information becomes available, the mistakes in our concepts will be discarded and those hypotheses which stand up to the experimental confrontation will serve as a basis for further and more accurate hypotheses. In this discussion, we have emphasized the biochemical events at the intracellular level and a few of the basic underlying constructs relating to the cleft palate from our modern biochemical knowledge of cellular and tissue differentiation.

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