CHAPTER 21

MOLECULAR STUDIES OF FACIAL CLEFTING: FROM MOUSE TO MAN

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21.1 INTRODUCTION

The human lip and palate form as a result of the cell proliferation (growth), apposition, and fusion of embryonic facial processes between the 5th and 12th weeks of gestation. This requires that the processes appear in the correct place, achieve the correct shape and size, and have no obstruction to fusion. Given the complex nature of this oral development, we can readily imagine a long list of potential mishaps (Trasler and Fraser, 1977). Indeed, oral clefts are a major public health problem worldwide (Marazita et al., 1986; Melnick, 1992; Wyszynski et al., 1996) (Chapters 3 and 7).

Cleft lip with or without cleft palate (CL ± P) has an incidence at birth of about 1 in 500–1000 that varies by population; people of Asian descent often are at higher risk than those of Caucasian or African descent (Marazita et al., 1986; Melnick, 1992; Murray, 1995; Wyszynski et al., 1996). In all populations there are significantly more males born with CL ± P than females. The incidence at birth for cleft palate alone (CP) is relatively uniform across populations at about 1 in 2000; significantly more females are born with CP than males (Shields et al., 1981; Wyszynski et al., 1996). It has clearly been established that CL ± P and CP are etiologically distinct (Melnick and Shields, 1982). People with CL ± P very rarely have relatives with CP and vice versa. What CL ± P and CP do share is that despite 50 years of intense study, the etiologies of both are largely an enigma.

21.2 ETIOLOGY OF NONSYNDROMIC ORAL CLEFTS

In 1875, Charles Darwin wrote: "Although many congenital monstrosities are inherited, of which examples have already been given, and to which may be added the lately recorded case of the transmission during a century of hare-lip with a cleft-palate in the writer's own family [Sproule, 1863], yet other malformations are rarely or never inherited. Of these latter cases, many are probably due to injuries in the womb or egg, and would come under the head of non-inherited injuries or mutilations." So the matter stood until the "discovery" of Mendel 25 years hence.

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At the outset of the 20th century in England, there arose a venomous dispute between Mendelian geneticists, such as Bateson at Cambridge, and anti-Mendelian biometricians, such as Pearson at the Galton Laboratory in London, over the genetic etiology of such "physical deformities" as cleft lip and palate (Melnick, 1997). To Pearson and colleagues, such traits were an expression of physical and racial degeneracy that could be traced to polygenically poor protoplasm. To Bateson and his associates, such traits were Mendelian unit characters whose segregation could be seen in carefully constructed family pedigrees. Bateson dismissed the work of the anti-Mendelians as "unsound in construction" and predicted such thinking would inevitably lead to "brutal" control of those who the larger society deemed unfit. History proved Bateson astutely prescient.

By 1925, there was a growing response to both sides of this argument. This is well represented by the writings of H. S. Jennings, Henry Walters professor of zoology and director of the Zoological Laboratory, Johns Hopkins University. In *Prometheus* (Jennings, 1925), he dismisses Pearsonism and takes on the shortcomings of the Bateson camp:

> These facts—the [so-called "unit characters" of Mendelism]—gave rise to a general doctrine, a philosophy of heredity and development, a doctrine which has had and still has a very great influence on general views of life. It is to this doctrine that the prevailing ideas as to the relation of heredity and environment, as to the relative powerlessness of environment, are due. But it has turned out to be a completely mistaken one. . . . The doctrine is dead—though as yet, like the decapitated turtle, it is not sensible of it. . . . What recent investigation has shown is this: the [genes] interact, in complex ways, for long periods; and every later characteristic is a long-deferred and indirect product of this interaction. Into the production of any characteristic has gone the activity of hundreds of the genes . . . ; and many intermediate products occur before the final one is reached. . . . The genes then are simply chemicals that enter into a great number of complex reactions, the final upshot of which is to produce the completed body. . . . In, producing the structures [nerve, muscle, bone, gland, and other tissues], the genes interact, not only with each other, with the cytoplasm, with the oxygen from the surrounding medium, and with the food substances in the cytoplasm; but also, what is most striking and important, with products from the chemical processes in neighboring cells. . . . What any given cell shall produce, what any part of the body shall become; what the body as a whole shall become—depends not alone on what it contains—its "heredity"—but also on its relation to many other conditions; on its environment.

This early explication of the epigenotype, a series of interrelated developmental pathways through which the adult form is realized (King and Stansfield, 1990), informed Jennings's understanding of the etiology of complex human diseases. He wrote:

> If a characteristic is observed in a given case to be inherited as a sex-linked character, we cannot be certain that it will be sex-linked in other cases. If it is recessive in some stocks, it may be dominant in others. . . . [H]undreds of genes are required to make a mind—even a feeble mind. . . . Doubtless feeble-mindedness is produced in hundreds of different ways—some sorts heritable according to one set of rules, others according to other sets of rules. . . . It is a commonly received dogma that if two parents are defective in the same hereditary characteristic, all the offspring will have this defect. But this need not occur. It will be true only if the defective characteristic is due to a peculiarity of the same gene in the two parents. Where it is due to defects in different genes in the two parents [genetic hetero-

> geneity], then the latter supplement each other, and none of the offspring have the defective feature. . . . Heredity is not the simple, hard-and-fast thing that old-fashioned Mendelism represented it. Further, more attentive observation has revealed that any single one of the genes affects, not one characteristic only, but many [pleiotropy]. . . . The idea of representative hereditary units, each standing for a single later characteristic, is exploded; it should be cleared completely out of mind.

Hyperbole aside, the kernal of Jennings's argument is that complex human traits (organ formation, mentation, etc.) are the result of tissue-specific epigenotypes, and these are related to multigenic inheritance and gene–environment interactions. Lip and palate formation are but two examples of this.

In 1942, Poul Fogh-Andersen published his seminal study of hundreds of CL ± P and CP families. He concluded that oral clefts are Mendelian autosomal-dominant disorders with greatly reduced penetrance. In other words, they were Mendelian/Batesonian "unit characters." A few years later, Curt Stern (1949) reached a different conclusion from the published data: "*Harelip* and *cleft-palate* are developmental abnormalities which have a genetic basis. In many pedigrees, they depend on the cooperation of specific alleles at several autosomal loci, and, in addition, require the presence of mostly uncontrolled environmental factors." Stern's conclusion was consistent with the epigenetic model put forth by Jennings (1925). Two decades later, the multifactorial/threshold model (MF/T) was introduced to the problem (Carter, 1969). The MF/T model provided a clever and innovative first approximation to a solution of what was clearly a difficult problem: it recognized that the genetic component was likely non-Mendelian and that environment played an important role as well. The MF/T model logically gave rise to a series of testable predictions of population and family data. Unfortunately, these predictions were rarely, if ever, satisfied when subjected to statistical analysis (Marazita et al., 1992; Nemana et al., 1992; Melnick, 1992; Wyszynski et al., 1996).

Since the mid-1970s, other etiologic models have been proposed (see review by Melnick, 1992): (1) single-gene inheritance of environmental susceptibility, (2) stochastic single-gene inheritance, (3) pure chance, (4) allelic restiction, and (5) emergenic inheritance. In essence, all of these are but elaborations (mathematical and otherwise) on Stern's insight from more than 50 years ago. From the weight of the current evidence, it is clear that there are important major gene effects (Melnick, 1992; Murray, 1995; Wyszynski et al., 1996); these tentatively appear to involve genes related to growth or fusion of facial processes (Lidral et al., 1998; Machida et al., 1999; Tanabe et al., 2000). Nevertheless, the inheritance patterns of CL ± P and CP are not classically Mendelian, exhibiting phenocopies, incomplete penetrance, genetic heterogeneity within and between populations, and the influence of modifier genes and diverse environmental factors such as folic acid deficiency (Loffredo et al., 2001; Martinelli et al., 2001; Piedrahita et al., 1999) and corticosteroid exposure (Park-Wyllie et al., 2000). This is well illustrated by the Fraser-Juriloff paradigm (Fraser, 1980) of differences in susceptibility to an environmental teratogen resulting from a genetically determined difference in *normal* oral development (Figure 21.1).

21.3 INVESTIGATING THE FRASER-JURILOFF PARADIGM

The elucidation of genetic factors for complex etiologies, such as those for oral clefts, is proving to be frustrating. There have been many reports of genes or loci that might be linked or associated with clefting, but none have been unequivocal

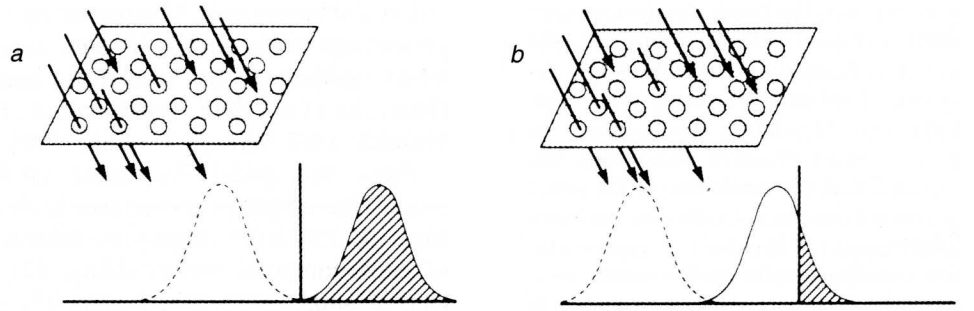

Figure 21.1 Fraser-Juriloff model of CP susceptibility. The roof with holes in it represents the maternal barrier between teratogen (arrows) and embryo. The x-axis represents the phenotypic distribution, normal to the left of the vertical threshold and abnormal to the right; the threshold separates palate closure from palate nonclosure. (a) Palate closure is normally late (*slow growth*), so the phenotypic distribution for this genotype (dashed curve) is near the threshold, and the delaying effect of the teratogen causes all embryos (solid curve) of this genotype to fall beyond the threshold and be affected (hatched area). (b) In an early closing (*faster growth*) genotype, the same delay causes a minority of embryos to be affected. Of course, these two cases are the outer boundaries of the model, and there will be many genotypes (dashed curves) at varying distances to the left of the threshold (Fraser, 1980).

(Ardinger et al., 1989; Hecht et al., 1991; Lidral et al., 1998; Machida et al., 1999; Tanabe et al., 2000). The modest nature of the identified gene effects for oral clefts likely explains the contradictory and inconclusive claims about their identification (Melnick, 1992; Murray, 1995). Despite the small effects of such genes, the magnitude of their attributable risk (the proportion of people affected due to them) may be large because they are quite frequent in the population (Melnick, 1992; Risch and Merikangas, 1996). Knowing this does not lessen the frustrations so far encountered in the search for etiologic solutions to human clefting.

Molecular geneticists looking for ways to understand human disease have increasingly turned to the mouse as biomedicine's model mammal (Malakoff, 2000). However, even the search for mouse models of nonsyndromic oral clefting has proved frustrating, at least as an attempt to confirm or inform the human data. Transforming growth factor-α (*TGFA*) was purported to be associated with nonsyndromic clefting (Ardinger et al., 1989). Not only has this not been confirmed in humans (Hecht et al., 1991; Lidral et al., 1998; Machida et al., 1999) but *Tgfa* knockout mice do not exhibit a cleft phenotype (Luetteke et al., 1993). Knockout of the receptor for Tgfa (Egfr) results in syndromic clefting, including micrognathia and other facial abnormalities (Miettinen et al., 1999). Transforming growth factor-β3 (*TGFB3*) and the transcription factor *MSX1* were also purported to be associated with CL ± P, and *MSX1* with CP, in humans (Lidral et al., 1998). Again, this has not been confirmed (Tanabe et al., 2000). Further, while *Msx1* and *Tgfb3* knockout mice exhibit clefting, the clefting is *syndromic* (Kaartinen et al., 1995; Proetzel et al., 1995). Similarly, while human nonsyndromic CL ± P has been significantly associated with *TGFB2* (Tanabe et al., 2000), *Tgfb2* knockout mice exhibit *syndromic* clefting, including cardiac, lung, limb, spinal, eye, urogenital, and other craniofacial defects (Sanford et al., 1997).

Human linkage/association studies and mouse studies of transgenic models have provided limited insight into the etiology of oral clefting. Considering Stern's (1949) modeling of the human data and the

Fraser-Juriloff paradigm (Fraser, 1980), it is little wonder that there is uncertainty. Clearly we have far more work to do in mouse studies, searching for more embryologically and genetically appropriate analogies and homologies before we return to human studies. The central problem is determining what is signal and what is noise by understanding what detail at the level of individual units is essential to understanding more macroscopic regularities (Levin et al., 1997). In this regard, it is instructive to recount the emerging story of CP susceptibility in H-2 congenic mice, if only as a "proof" of a 25-year-old paradigm (Melnick and Shields, 1976).

21.3.1 H-2 Haplotype: Maternal and Embryonic Effects

The exposure of embryonic mice to corticosteroids (CORT) has long been known to result in CP (Fraser and Fainstat, 1951). Studies in our laboratory, and others, have shown consistently that CORT-induced CP is related to genetic variation at or near the H-2 complex on mouse chromosome 17 (Melnick et al., 1981a; Goldman, 1984; Gasser et al., 1991). H-2 congenic mice were originally developed by George Snell in the late 1940s. They share identical genetic backgrounds, with the exception of a 3–18 cM region of chromosome 17 (i.e., the congenic region), which encompasses the H-2 complex and defines each H-2 haplotype (Fig. 21.2) (Vincek et al., 1990). These mice are an important tool for investigating the contribution of specific congenic genes to develoment, including palate morphogenesis.

By using the H-2 congenic mice, we have shown that B10.A ($H\text{-}2^a$) mice are ninefold more susceptible to CORT-induced CP than B10 ($H\text{-}2^b$) mice (Table 21.1); reciprocal hybrid studies have demonstrated a significant maternal effect (Table 21.1) (Melnick et al., 1981a). In reciprocal crosses between two inbred strains, the two types of F_1 females are genetically identical. If two inbred strains differ in response to a teratogen, one being susceptible and the other resistant, we can test for cytoplasmic effects by backcrossing the two types of reciprocal F_1 females to the susceptible strain males (Fig. 21.3) (Melnick et al., 1983). If there are statistically significant differences in the frequency of developmental abnormality in the two types of treated backcross offspring (in the direction of the line of the mother's mother), a genetic difference in maternal physiology is ruled out as a reasonable explanation and a cytoplasmic factor (mitochondrial genes) is suggested (Biddle and Fraser, 1977). In fact, there is a significant increase in developmental anomaly frequency (DAF) when the mother's mother is of the susceptible B10.A strain (Fig. 21.3) (Melnick et al., 1983).

One explanation for this interesting finding is the recently demonstrated interaction between nuclear and mitochondrial

TABLE 21.1 CORT-Induced Cleft Palate in H-2 Congenic Mice

Cross Dam × sire	CORT mg/kg	Embryo (haplotype)	Cleft Palate Frequency (%)
B10.A × B10.A	0	B10.A ($H\text{-}2^a/H\text{-}2^a$)	0
B10.A × B10.A	2	B10.A ($H\text{-}2^a/H\text{-}2^a$)	45
B10 × B10	0	B10 ($H\text{-}2^b/H\text{-}2^b$)	0
B10 × B10	2	B10 ($H\text{-}2^b/H\text{-}2^b$)	5
B10.A × B10	2	B10.A.B10 ($H\text{-}2^a/H\text{-}2^b$)	31
B10 × B10.A	2	B10.B10.A ($H\text{-}2^b/H\text{-}2^a$)	21

Significance tests: (1) B10.A (2 mg/kg) vs. B10 (2 mg/kg): $p < 0.001$; (2) B10.A.B10 vs. B10.B10.A: $p < 0.05$.

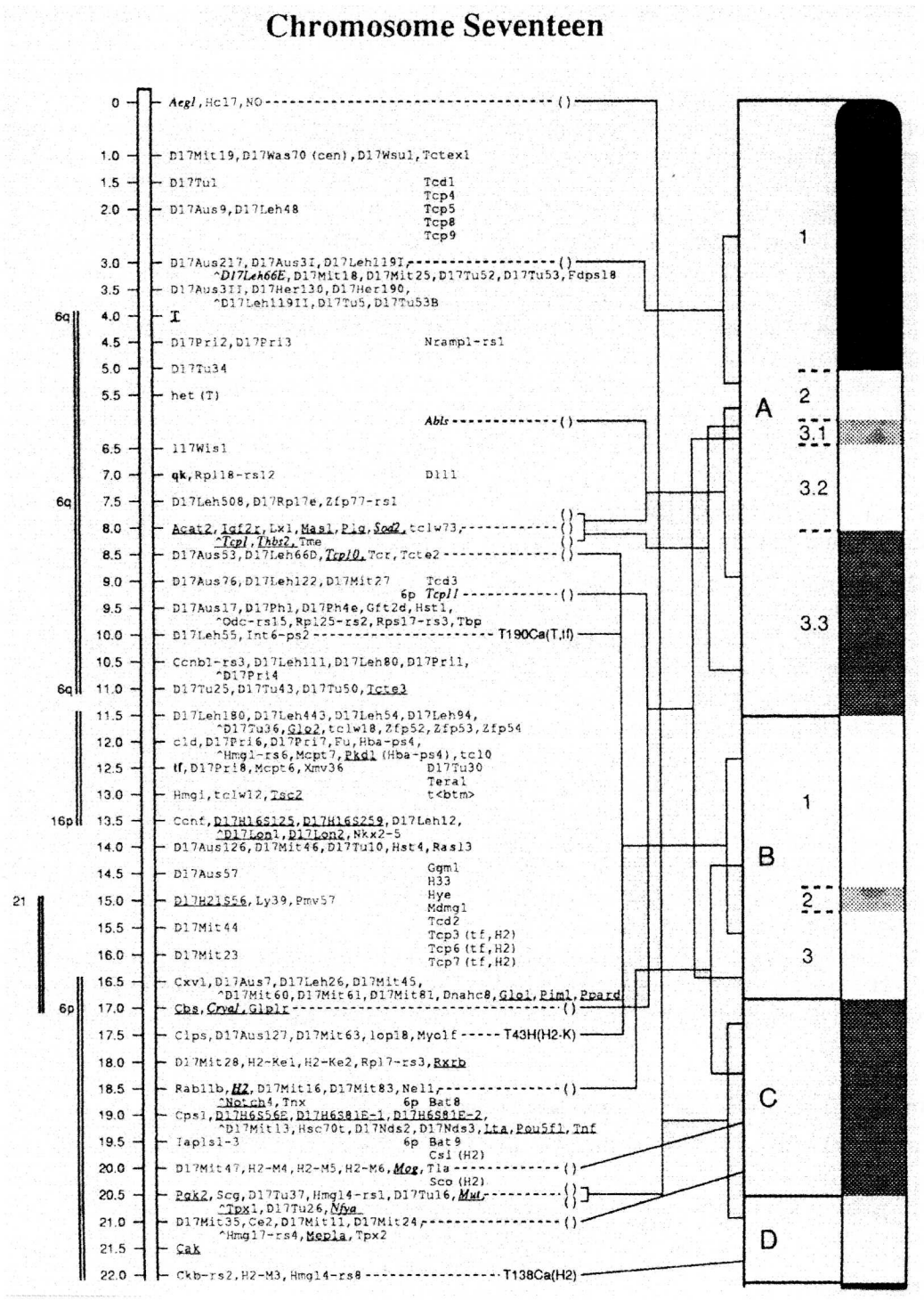

Figure 21.2 Mouse gene map, chromosome 17 (Mouse Gene Informatics—Jackson Laboratory: www.informatics.jax.org). Note the positions of *Igf2r* and *Plg* at 8.0 cM, as well as *H2* at 18.5 cM (all of which have corresponding genes on human chromosome 6).

genomes (Johnson et al., 2001). In 1982, Ferris and co-workers examined mouse mitochondrial DNA from various locales in the Northern Hemisphere using restriction enzymes that cut the molecule at an average of 150 sites and found a high level of restriction-site polymorphism in wild mice but no variation among the "old" inbred strains commonly found in the laboratory, including the C57BL strain, which is the background of the H-2 congenic mice described above. Ferris and colleagues (1982) suggest that all the "old" inbred strains of laboratory mice are descendants of one single female who lived sometime between 1200 B.C. and A.D. 1920. Perhaps what is seen in the experiments outlined in Figure 21.3 is the phenotypic effects of "gene-gene interaction" between variant congenic genotypes and an invariant mitochondrial genotype. The key, then, is finding the relevant gene(s) in the congenic genotypes.

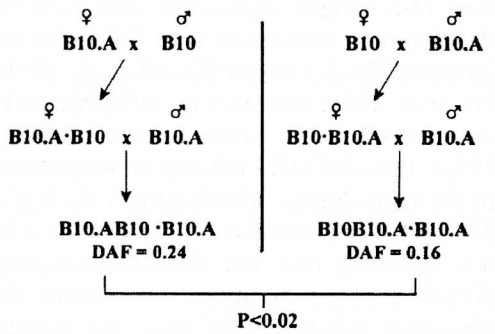

Figure 21.3 Testing for a cytoplasmic factor (mitochondrial genes). Reciprocal F_1 females are backcrossed to males of the susceptible strain (B10.A). There is a significant difference in the frequency of developmental abnormality (DAF) in the direction of the line of the mother's mother, and, thus, evidence of a cytoplasmic (mitochorial) effect.

21.3.2 Corticosteroids and Gene Regulation

Corticosteroid hormone signaling is somewhat unique among signal transduction mechanisms (Fig. 21.4). CORT is lipophilic and crosses the cell membrane, where it

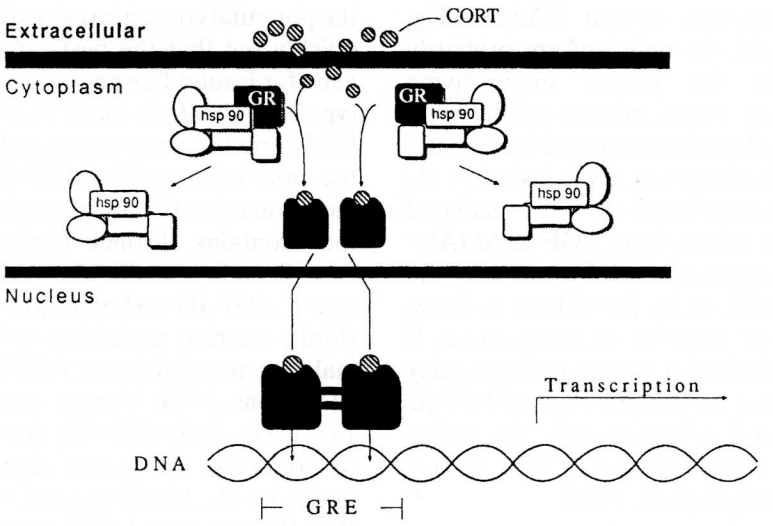

Figure 21.4 Corticosteroid signal transduction pathway. Lipophilic glucocorticoid (CORT) translocates across the plasma membrane to the cytoplasm and binds to the glucocorticoid receptor (GR). Activation of GR releases the hsp90-dominated complex to which it is bound. CORT/GR translocates to the nucleus, homodimerizes, and binds to a DNA glucocorticoid response element (GRE) in the regulatory region of target genes to up- or downregulate transcription.

binds to its cognate cytoplasmic receptor (GR). Activated ligand-bound receptor is translocated to the nucleus, dimerizes with other ligand-bound receptors, and binds to response elements (GREs) in the regulatory region of target genes. In essence, the CORT/GR complex serves as a transcription factor, up- and downregulating gene expression.

The TGF-β family of proteins is involved in regulating cell proliferation, differentiation, and extracellular matrix formation and degradation (Dunker and Krieglstein, 2000). The three mammalian TGF-β isoforms are TGF-β1, TGF-β2, and TGF-β3, each encoded by different genes on different chromosomes. There is a significant increase in TGF-β1 and TGF-β3 transcript levels and a significant decline in TGF-β2 transcript levels with progressive palatal development (Jaskoll et al., 1996). All TGF-β isoforms signal via the same cell membrane–bound heteromeric receptor complex: TGF-β receptor type I and ligand binding TGF-β receptor type II (Fig. 21.5). Signal transduction from the receptor to the nucleus is mediated by intracellular effector molecules termed SMADs (Fig. 21.5). TGF-β2, the only isoform primarily localized in the palatal mesenchyme (Jaskoll et al., 1996), inhibits palatal mesenchymal cell proliferation and thus palatal shelf growth (Ferguson, 1988; Jaskoll et al., 1996). Downregulation of Cdk4-mediated cell division results from TGF-β2/SMAD-induced upregulation of the transcription factor p27 (Fig. 21.5). For palates to grow, then, TGF-β2 must be downregulated. It has been shown that CORT-induced delay in the normal downregulation of TGF-β2 transcription is a key event in the pathogenesis of CORT-induced CP in B10.A embryos (Jaskoll et al., 1996).

21.3.3 The TGF-β/IGF Connection

H-2 haplotype-specific differences in the rate of embryonic development in B10.A and B10 congenic mice have been studied extensively. Significant strain differences in the number of embryonic day 12 (E12) embryos that reach the appropriate Theiler developmental stage (Theiler, 1989) are seen routinely (Fig. 21.6). In addition, B10.A mice produce smaller embryos, with delayed palatal development, lung maturation, H-2 antigen expression, and skeletal development compared with B10 mice at identical Theiler stages (Good et al., 1991; Hu et al., 1990; Jaskoll et al., 1991; Melnick and Jaskoll, 1992; Melnick et al., 1981b, 1982). Thus, if CORT inhibits palatogenesis to the same degree in both strains via TGF-β2 regulation (Jaskoll et al., 1996), then it is not suprising that the slower-developing B10.A embryo is more vulnerable to abnormal palatogenesis than the faster-developing B10 embryo, as predicted by the Fraser-Juriloff paradigm (Fig. 21.1). The question is what is the link between genes in the 3–18 cM congenic region of chromosome 17 and TGF-β2, the gene for which is on chromosome 1 and thus identical in B10.A and B10 mice.

Viewing the map of chromosome 17 in the potential congenic region (Fig. 21.2), we quickly see that the best candidate is the gene for insulin-like growth factor receptor type 2 (*Igf2r*). *Igf2r* maps to approximately 8 cM from the centromere and 10 cM from the more telomeric H-2. IGF-IIR is a large, membrane-bound glycoprotein (~300 kDa) that contains distinct binding sites for two ligands: insulin-like growth factor type 2 (IGF-II) and mannose-6-phosphate (M6P)–bearing molecules such as lysosomal enzymes and latent TGF-β (Jones and Clemmons, 1995; Vignon and Rochefort, 1992) (Fig. 21.5). IGF-IIR does not appear to transduce mitogenic signals (Moats-Staats et al., 1995); instead, it sequesters IGF-II from type I IGF receptors, which mediate IGF-II growth signal transduction (Ballard et al., 1986; Lau et al., 1994; Wang et al., 1994; for reviews, see Barlow, 1995, and Haig and Graham, 1991). This seques-

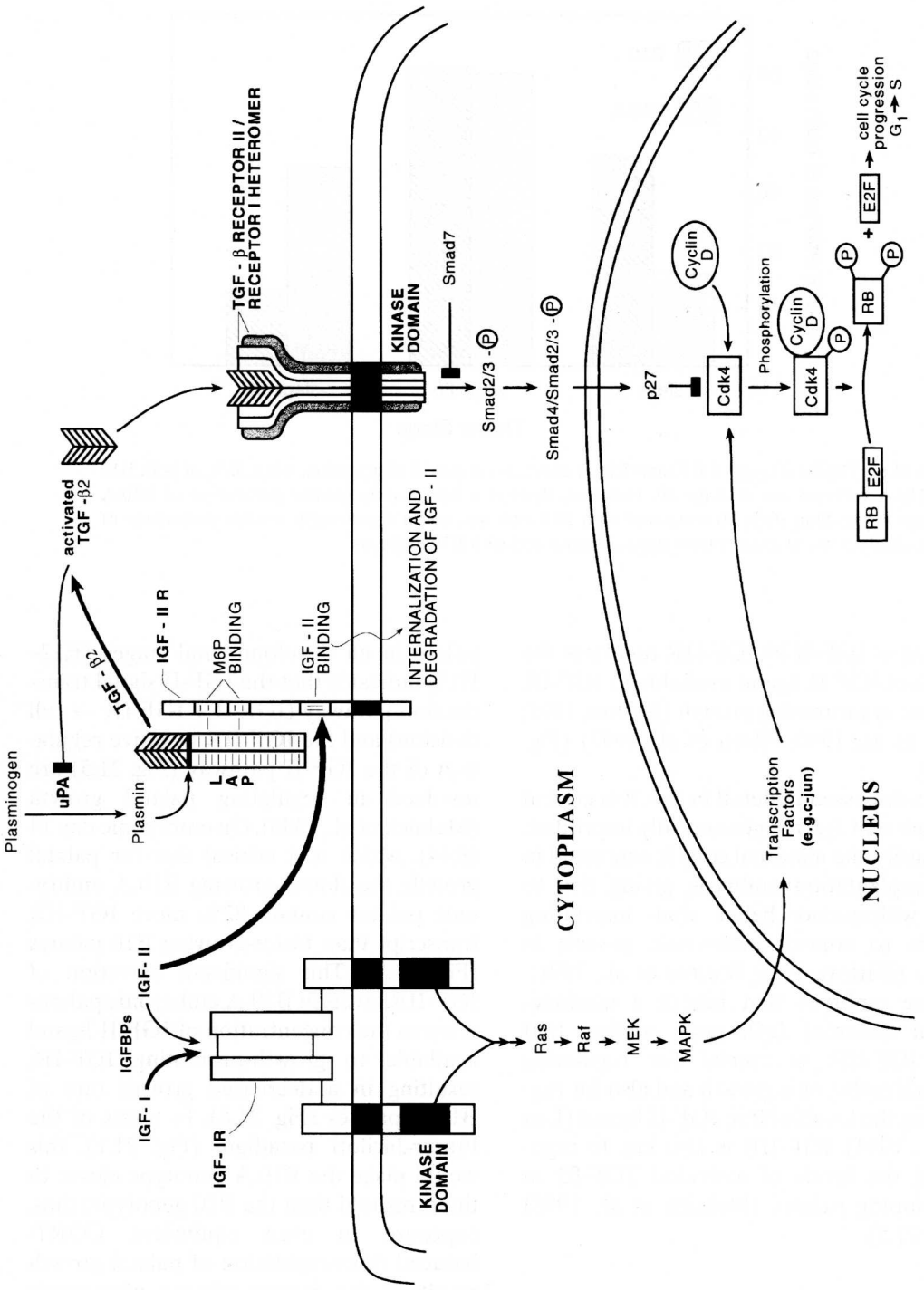

Figure 21.5 Epigenetic network of information processing relative to cell proliferation. IGF, insulin-like growth factor; R, receptor; TGF-β, transforming growth factor-beta; LAP, latency-associated protein; M6P, mannose-6-phosphate; uPA, urokinase-type plasminogen activator; Ras, a G protein; Raf, Ras-activated factor; MEK, a protein kinase; MAPK, mitogen-activated protein kinase; Smad, a family of transcription factors; p27, a transcription factor.

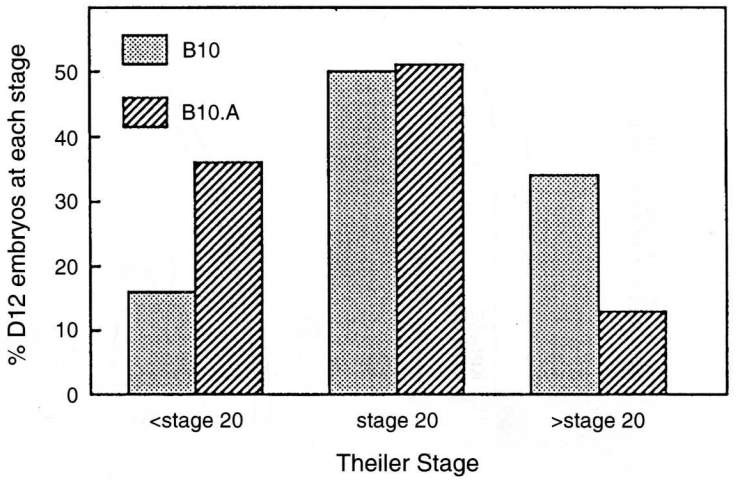

Figure 21.6 Theiler staging of B10 and B10.A embryos on day 12 of gestation, when 50% of both B10 and B10.A embryos are at stage 20. However, there is a significantly greater percentage of B10.A embryos at less than stage 20 compared with B10 embryos, and a significantly smaller percentage of B10.A embryos are at greater than stage 20 compared with B10 embryos.

tration of IGF-II by IGF-IIR regulates the levels of IGF-II ligand available to IGF-IR for use in promoting growth (Barlow, 1995; Ellis et al., 1996; Filson et al., 1993) (Fig. 21.5).

As discussed in detail below, it is critical to note that *Igf2r* is genomically imprinted. Primarily, the maternal copy is expressed in postimplantation embryos, giving rise to the widespread belief that imprinting serves to control embryonic growth in utero (Barlow, 1995; Barlow et al., 1991). Mouse embryos that inherit a nonfunctional maternal *Igf2r* gene confirm that the IGF-IIR is crucial for regulating normal embryonic growth and also for regulating the levels of free IGF-II ligand (Lau et al., 1994). IGF-IIR is also key to regulating the levels of activated TGF-β2 in developing palates (Melnick et al., 1998) (Fig. 21.5).

21.3.4 IGF and Palate Morphogenesis

The presence of IGF-II, IGF-IR, and IGF-IIR in all cellular types of the embryonic palate in all developmental stages (E12–E15) indicates that the IGF-II signal transduction pathway (IGF-II + IGF-IR → cell division) and the IGF-IIR negative regulation of the IGF-II pathway (Fig. 21.5) are involved in regulating palatal growth (Melnick et al., 1998). On embryonic day 14 (E14), which is a critical day for palatal growth, the slower-growing B10.A embryonic palates contain 82% more IGF-IIR transcript than faster-growing B10 palates (Fig. 21.7). This significant elevation of IGF-IIR levels in B10.A embryonic palates reduces the concentration of IGF-II ligand available to growth-promoting IGF-IR, resulting in a decreased growth rate of B10.A palates (Fig. 21.8). In terms of the Fraser-Juriloff paradigm (Fig. 21.1), this would place the B10.A genotype closer to the threshold than the B10 genotype; thus, exposure to even equivalent CORT-induced downregulation of palatal growth results in far greater adverse phenotypic outcomes for B10.A than for B10 embryonic palates, as previously noted (Table 21.1) (Melnick et al., 1981a).

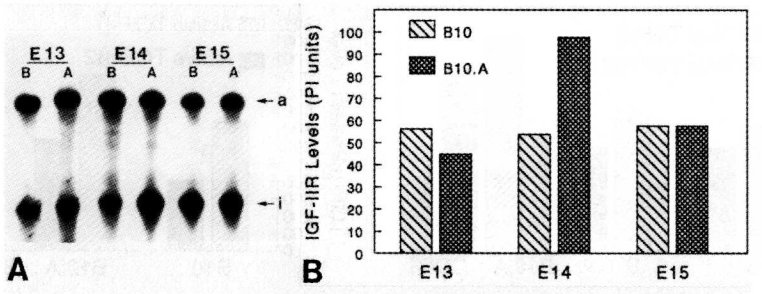

Figure 21.7 IGF-IIR transcript levels in developing B10 and B10.A palates. (A) An Rnase protection assay to compare the steady-state levels of IGF-IIR mRNA in E13, E14, and E15 B10(B) and B10.A(A) palates. (B) Bars represent mean phosphor imaging (PI) units of IGF-IIR mRNA; E14 B10.A levels are 82% greater than E14 B10 ($p < 0.05$), while all other mean levels are equivalent (Melnick et al., 1998).

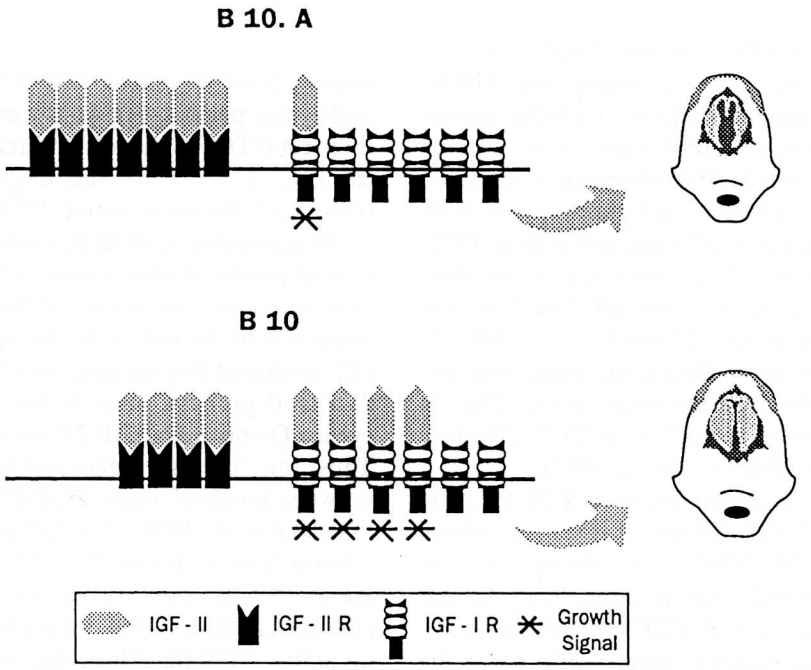

Figure 21.8 Schematic of the IGF-IIR role in palate development. IGF-II binds to IGF-IR and IGF-IIR, having a higher affinity for IGF-IIR than for IGF-IR. The lower level of IGF-IIR in the E14 B10 palate results in increased availability of IGF-II, thereby enabling more of the ligand to bind the IGF-IR in B10 palates compared with B10.A palates. Because only IGFII/IGF-IR binding transduces a mitogenic signal (asterisks), the result is an accelerated rate of morphogenesis in the B10 palate compared with the B10.A palate.

21.3.5 IGF-IIR and TGF-β2 Activation

All the mammalian TGF-βs are 25 kD homodimers in their biologically active form; they show a high level of sequence conservation (Lawrence, 1996). The larger, latent TGF-β is comprised of one mature TGF-β molecule noncovalently bound to the proregion dimer, the latency-associated peptide (LAP) (Fig. 21.5); TGF-β1 and

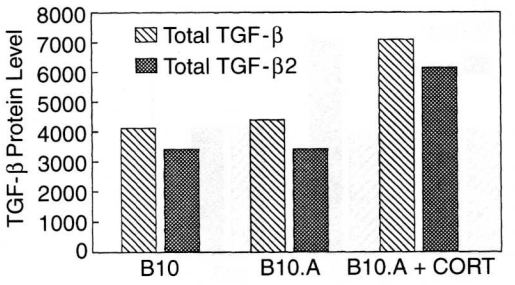

Figure 21.9 Mean total TGF-β and total TGF-β2 protein levels in E14 palates. There is no substantial difference between strains in total TGF-β levels; CORT treatment induces a 62% increase in total TGF-β levels and a 56% increase in total TGF-β2 levels (Melnick et al., 1998).

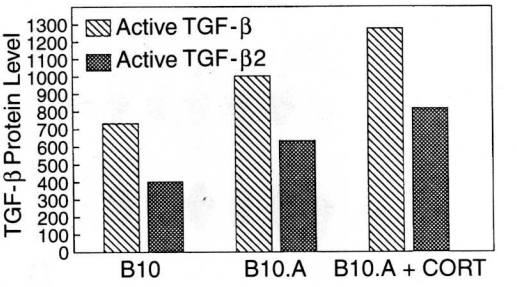

Figure 21.10 Mean total active TGF-β and total active TGF-β2 protein levels in E14 palates. B10.A palates exhibit a 37% greater level of active TGF-β than B10 and a 57% greater level of active TGF-β2; elevated CORT levels significantly increase the levels of activated TGF-β, 64% of which is TGF-β2 (Melnick et al., 1998).

TGF-β2 LAPs contain M6P residues (Brunner et al., 1992; Dennis and Rifkin, 1991; Gleizes et al., 1997). Cellular activation of latent TGF-β appears to require binding to the M6P binding site of the IGF-IIR and is plasmin and plasminogen activator dependent (Dennis and Rifkin, 1991; Gleizes et al., 1997). Plasminogen and plasminogen activators are all found in the embryonic palate (Melnick et al., 1998). It is probably more than coincidence that the polymorphic plasminogen gene, *Plg*, is closely linked to *Igf2r* (Fig. 21.2) (Barlow et al., 1991; Friezner et al., 1990).

The relationship between TGF-β2 activation and IGF-IIR is key. Because embryonic day 14 (E14) is a critical stage of palatal growth that is dependent on the downregulation of TGF-β2 (Jaskoll et al., 1996), and known strain differences in growth rate (Melnick and Jaskoll, 1992) are associated with TGF-β2 (Jaskoll et al., 1996) and IGF-IIR (Melnick et al., 1998) expression, it is not surprising that greater availability of the IGF-IIR receptor in B10.A embryonic palates (Fig. 21.7) would result in a higher level of activated TGF-β2 (Melnick et al., 1998). Approximately one-fifth of the total TGF-β is activated, and approximately two-thirds of the total activated TGF-β is TGF-β2 (Figs. 21.9 and 21.10). It is particularly noteworthy that E14 B10.A embryonic palates have a 57% greater level of active TGF-β2 than B10 embryonic palates (Fig. 21.10), even though their total TGF-β2 levels are nearly identical (Fig. 21.9). Thus, the more IGF-IIR (receptor), the more active TGF-β2.

As noted above, TGF-β2-induced inhibition of palatal mesenchymal cell proliferation is related to arrest of the $G_1 \rightarrow S$ transition of the cell cycle through SMAD/p27-mediated downregulation of Cdk4 (Fig. 21.5) and, perhaps, other G_1 factors such as cyclins D and E and Cdk2 (Derynck, 1994). Thus, an inverse relationship is seen between levels of active TGF-β2 and Cdk4 (Melnick et al., 1998). E14 B10 palates with a lower level of active TGF-β2 (Fig. 21.10) have a 52% greater level of Cdk4 transcript (Fig. 21.11) than B10.A with a higher level of active TGF-β2. Thus, the variation of TGF-β2/IGF-IIR-mediated growth inhibition in the late G_1 phase of the cell cycle (Figs. 21.5 and 21.7–21.11) (Melnick et al., 1998) appears to account for the slower growth and development of B10.A embryonic palates relative to its B10 congenic partner (Melnick and Jaskoll, 1992).

21.3.6 CORT-Induced Palate Pathogenesis

It is well established that CORT induces CP many-fold in B10.A embryos relative to

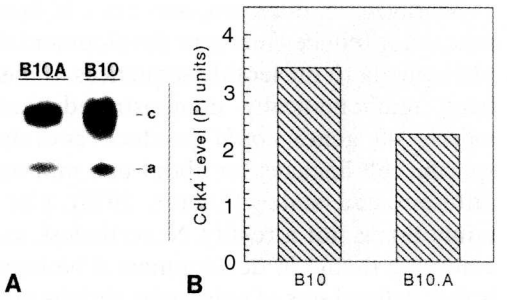

Figure 21.11 Cdk4 mRNA levels. (A) An Rnase protection assay was used to compare the steady-state levels of Cdk4 mRNA in E14 B10 and B10.A palates. (B) Bars represent E14 palatal mean phosphor imaging (PI) units of Cdk4 mRNA; B10 palates exhibit a significant 52% increase in Cdk4 mRNA levels compared with B10.A palates (Melnick et al., 1998).

its B10 congenic partner when exposed on E12 (Melnick et al., 1981a). CORT exposure has been shown to inhibit palatal mesenchyme cell proliferation, resulting in smaller palatal processes and CP (Potchinsky et al., 1996; Salomon and Pratt, 1979). CORT also delays by 1 day the downregulation of palatal TGF-β2 transcription normally seen on E14 (Jaskoll et al., 1996). Further, in E14 B10.A palates, elevated CORT exposure significantly increases TGF-β protein levels, 87% of which is TGF-β2, as well as the levels of active TGF-β, 64% of which is TGF-β2 (Figs. 21.10 and 21.11) (Melnick et al., 1998). This enhances the TGF-β2/IGF-IIR-mediated growth inhibition via downregulation of Cdk4 in late G_1 of the cell cycle.

Thus, we have an outline of the pathogenetic mechanism in B10.A embryos: Slower-growing B10.A embryos have an upregulation of IGF-IIR that serves to sequester IGF-II from the growth-promoting IGF-IR and to bind more CORT-upregulated, latent TGF-β2 for subsequent plasmin-dependent activation. Higher levels of TGF-β2 signaling lead to palatal growth inhibition at a critical stage of palatogenesis and, thus, subsequent CP. In terms of the Fraser-Juriloff paradigm (Fig. 21.1), B10.A embryos that are already close to the threshold of abnormality are pushed beyond that threshold with the CORT-induced upregulation of activated TGF-β2.

21.4 IGF-IIR/TGF-β2 EPIGENETIC NETWORK

Figure 21.5 is a model of information processing as it relates to cell proliferation of mesenchyme in embryonic palates. Ligand-receptor binding is the first step in pathways of signal processing that effect specific gene expression and phenotypic change. Typically signaling pathways are studied as though information processing were linear. However, it is becoming increasingly apparent that pathways interact with one another, and the final biologic response is shaped by this interaction (Bhalla and Iyengar, 1999; Strohman, 1997). This results in signaling *networks* of great complexity and nonlinearity. Such networks are *epigenetic* networks in that they include feedback to the genome and changing patterns of gene expression (Strohman, 1997).

Although this representation of the molecular control of cell proliferation (Fig. 21.5) is not strictly reducible to its parts (labeled boxes, ovals, arrows, etc.), some of the known factors that make this complex epigenetic network nonlinear and adaptive include (1) IGF-IIR binds IGF-II with a very significantly greater affinity than IGF-IR (Jones and Clemmons, 1995); (2) although ligand binding of the IGF-IR is not the *sine qua non* for cell cycle progression, it is probably required for the cell cycle to be maintained at a normal rate (LeRoith et al., 1995); (3) since IGF-II and MP6-bearing molecules (e.g., latent TGF-β2) competitively bind to their cognate IGF-IIR sites because of steric hinderance or conformational change, any imbalance in ligand(s) and receptor concentration is likely to alter associated biological functions, such as IGF-II degradation, IGF-II/IGF-IR binding, and

TGF-β2 binding and activation (Vignon and Rochefort, 1992); (4) TGF-β decreases the mRNA expression of both uPA and tPA plasminogen activators and may stimulate PA inhibitor production (Agrawal and Brauer, 1996; Keski-Oja et al., 1988); (5) plasmin-dependent activation of TGF-β is modulated by surface localization of uPA by its recptor (Odekon et al., 1994). Functionally, then, the IGF-IIR/TGF-β2 epigenetic network is a dynamical network that uses a continuous logic to learn its rules from changing conditions. As such, it can be modeled mathematically as an artificial neural network.

21.4.1 Genes and Development

Nearly four decades ago, Maruyama (1963) clearly outlined the developmental biologist's nightmare:

> [I]t is not necessary for the genes to carry all the information regarding the adult structure, but it suffices for the genes to carry a set of rules to generate the information.
>
> The amount of information to describe the resulting pattern is much more than the amount of information to describe the generating rules and the positions of the initial tissues. The pattern is generated by the rules and by the interaction between the tissues. In this sense, the information to describe the adult individual was not contained in the initial tissues at the beginning but was generated by their interactions.
>
> [I]t is in most cases impossible to discover the simple generating rules after the pattern has been completed, except by trying all possible sets of rules. When the rules are unknown, the amount of information needed to discover the rules is much greater than the amount of information needed to describe the rules. This means that there is much more waste, in terms of the amount of information, in tracing the process backwards than in tracing it forward.

Maruyama's dilemma, and ours, is that we cannot reduce emergent developmental phenomena to nucleotide sequences. It has been mathematically demonstrated that merely 40 genes *could* produce entirely specific cell lineages for about one million differentiated states (Gierer, 1973). Certainly this is not a reality. Nevertheless, an emerging theme in developmental biology is that defined sets of epigenetic circuits are used in multiple places, at multiple times, for similar and sometimes different purposes during organogenesis (Melnick and Jaskoll, 2000). In the context of palate development and the IGF/TGF-β epigenetic network, we might also benefit by looking briefly at several other emerging themes in developmental biology: cellular automata, differential methylation, and submolecular information processing.

21.4.2 Game of Life

Important to the Fraser-Juriloff paradigm (Fig. 21.1) is the demonstration of significant measurable differences between inbred strains (or human individuals) for growth-related anatomic variables relevant to palate development. In support of this, Diewert (1982) finds significant quantitative differences between A/J and C57BL/6 inbred mice for several growth variables related to palatal shelf elevation, contact, and closure. These include the length of Meckel's cartilage relative to that of the bronchonasal cavity, the height and width of the oral cavity, and the width of the maxilla. Similar differences are seen in other inbred strain comparisons (Ciriani and Diewert, 1986), as well as in B10.A and B10 congenic mice (Melnick and Jaskoll, 1992). In sum, certain strains (A/J, B10.A) are slower growing, and ultimately somewhat smaller, than other strains (C57BL/6, B10).

We have seen above how this heterochronic palatal development is related to the IGF/TGF-β epigenetic network. To put

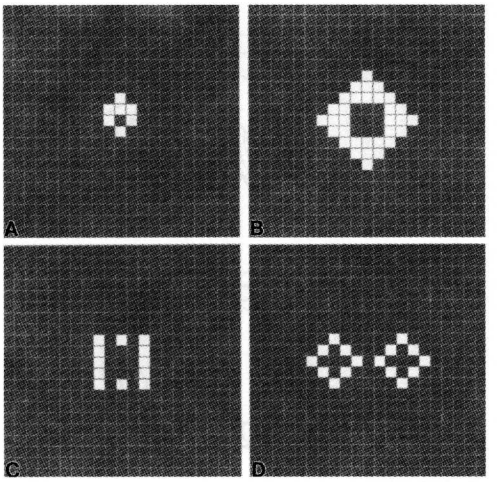

Figure 21.12 Game of Life. This is a cellular automaton that was invented by Cambridge mathematician John Conway. It consists of a collection of cells which, based on a set of simple rules, can live, die, or multiply; depending on the initial conditions, the cells form specific patterns from one generation to the next. (A) Initial condition "small exploder." (B) "Small exploder" seven generations later. (C) Initial condition "exploder." (D) "Exploder" seven generations later. (See www.bitstorm.org/game of life.)

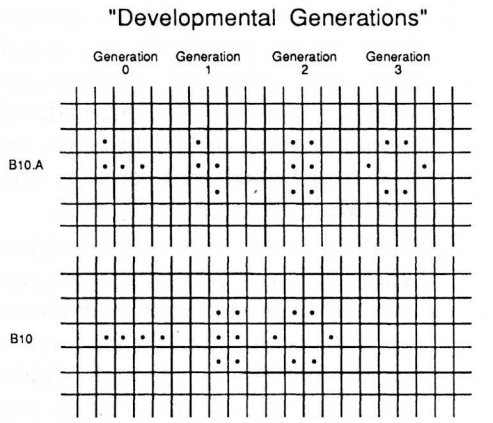

Figure 21.13 Conway's Game of Life and palatal development. Different initial conditions (patterns) can result in identical final stages, albeit over different time periods.

this relationship in a more amenable conceptual framework, it is convenient to call on John Conway's "Game of Life" (www.bitstorm.org/gameoflife/), a popular example of a two-dimensional cellular automaton. Using the same set of rules to determine the fate of future generations, it can be seen that any given initial condition (or starting pattern of occupied cells) will inevitably lead to a specific sequel pattern over any specified number of generations (Fig. 21.12). What is important to the present developmental problem is the property that a given configuration can have several preceding sequels due to several different initial conditions, but only one future.

Viewing Figure 21.13, it can be seen that different initial conditions (patterns) can lead to identical final states, albeit with different chronologies. While viewing a particular final state on a computer screen does not allow us to deduce its predecessors, the tools of developmental biology allow us to make a first approximation regarding organogenesis. Nevertheless, we have to always remember that the rules of the game are set by the epigenotype, a cellular informational system that integrates genetic and environmental information into a dynamical process able to generate responses that are functionally adaptive (Strohman, 1997). This is why the "Game of Life" (Fig. 21.12) is such an apt computational metaphor, and so informative.

Suppose strains B10.A and B10 are as represented in Figure 21.13; suppose the rules of the "game" are identical in each strain, and these are set by a well-conserved epigenotype; suppose the initial conditions (patterns) are influenced by IGF-IIR transcript and protein levels. Then we may look at Figure 21.13 in the following way:

1. Different initial conditions (phenotypes, "game patterns") are tightly associated with epigenetically determined differences in IGF-IIR levels in each strain (see Fig. 21.7 and methylation discussion below).

2. Different initial conditions (phenotypes, "game patterns") are associated with different chronologies; namely, B10.A requires three "developmental generations" to reach the final state, while B10 requires only two (Melnick and Jaskoll, 1992).
3. Identical final states (phenotypes, "game patterns") in both sequences (strains) have similar, if not identical, predecessor states (phenotypes, "game patterns"), though initial conditions (phenotypes, "game patterns") are quite different (Melnick, 1992).

From this list we can clearly visualize the larger organismal picture, namely that strain differences are largely chronologic differences, and these are determined by epigenetically mediated initial state (phenotype) differences that ultimately play their hand with a set rule book. It is tempting to think of this as knowledge, but game theory is a metaphor. Like most metaphors, it captures some aspects of the truth but leads us astray if we take it as anything but a first approximation.

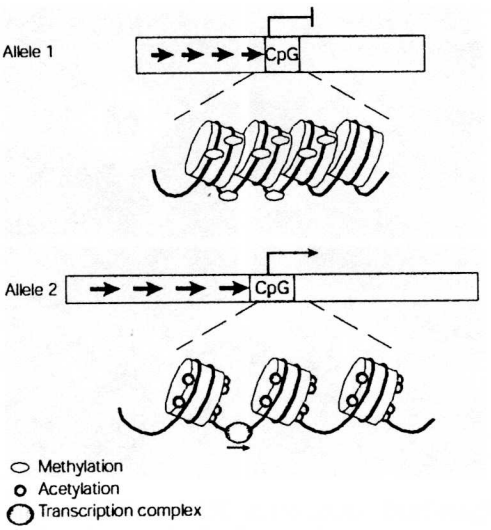

Figure 21.14 Imprinted genes. This is a diagramatic representation of a homologous pair of imprinted alleles (say the alleles for the gene *Igf2r*). Characteristics of imprinted genes such as CpG islands and repeats (arrows) are noted. The enlargement below each cognate chromosomal location illustrates the allele-specific epigenetic changes, such as nucleosomal condensation (via deacetylation) and methylation (allele 1) and increased nucleosomal spacing (via acetylation), demethylation, and the binding of a transcriptional complex for gene expression (allele 2). (*Source*: Adapted from Reik and Walter, 2001.)

21.4.3 IGF-IIR Imprinting and Methylation

Mammals exhibit the unique (and non-Mendelian) process of genomic imprinting. In this epigenetic process, a gene on one chromosome is silenced (imprinted), while its homologous allele on the other homologous chromosome is expressed. There are several known mechanisms of gene silencing, the best characterized being DNA methylation (see reviews by Pfeifer, 2000; Reik and Walter, 2001).

DNA methylation refers to the addition of methyl groups to cytosine residues of cytosine-guanine dinucleotide (CpG) repeats in the DNA sequence of specific genes. DNA methylation is mediated by several well-characterized methyl transferases. Gene silencing by methylation is associated with a characteristic change in chromatin structure (Fig. 21.14) (Razin, 1998; Wolffe, 1998). Methylation-associated allelic repression is usually quite stable, but it can be reversed (Ramechandani et al., 1999). DNA demethylase also shows CpG specificity, but it catalyzes the cleavage of a methyl residue from 5-methyl cytosine and its release as methanol. Thus, demethylase performs the reverse reaction to DNA methyltransferase and would seem to be a natural partner in shaping the site-specific methylation pattern of genomes, a *sine qua non* of normal mammalian embryogenesis (Li et al., 1992; Ramchandani et al., 1999).

Excluding X-chromosome inactivation (lyonization), the relatively small number

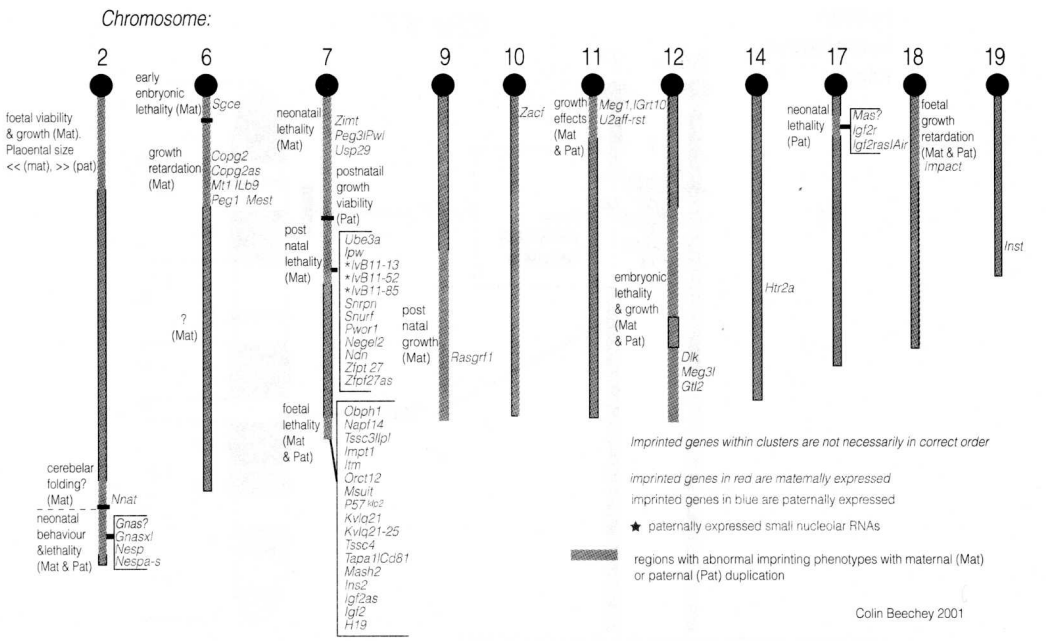

Figure 21.15 Mouse imprinted genes, regions, and phenotypes. (*Source*: From Beechey et al., 2001.)

of imprinted genes exhibit allelic expression differences that depend on parental origin (Fig. 21.15). Imprinted genes are found in clusters (Fig. 21.15), and these clusters are conserved between mouse and human (Fig. 21.16), leading to the proposition that the clustering of imprinted genes is essential to their regulation (Thorvaldsen and Bartolomei, 2000). Perusing the mouse genomic imprinting map (Fig. 21.15), it is easy to see how aberrant imprinting disturbs embryonic development. Such is certainly the case regarding *Igf2r*.

The significant increase in B10.A IGF-IIR is transient and specific to E14 (Fig. 21.7), a day that is critical to mouse palatal growth. The most likely mechanism is a switch from mostly monoallelic expression of *Igf2r* to more biallelic expression—a switch that results from more than relaxation of methylation (Lerchner and Barlow, 1997). Lerchner and Barlow show that the paternal *Igf2r* allele is repressed in mice from E6.5 onward; however, a low level of paternal expression remains in tissues that highly express the maternal allele from E7.5 onward. Functional polymorphism (monoallelic/biallelic) exists with the parental imprinting of the human *Igf2r* gene as well (Xu et al., 1993). Lerchner and Barlow propose that mere DNA methylation is not sufficient to cause monoallelic expression and must occur by a multifactorial process. This is supported by Smrzka and colleagues (1995), who found that the human *IGF2R* gene has the classic imprinting characteristics of monoparental methylation and replication asynchrony but does not show unequivocal monoallelic expression. Finally, additional genetic or epigenetic control of allelic expression is also supported by congenic mouse matroclinus reciprocal hybrid cross data (Melnick et al., 1998). B10.A/B10.A embryos had 60% greater IGF-IIR levels ($P < 0.01$) than B10.A/B10 embryos, which in turn were equivalent to B10/B10.A and B10/B10 embryos (Fig. 21.17). This can only

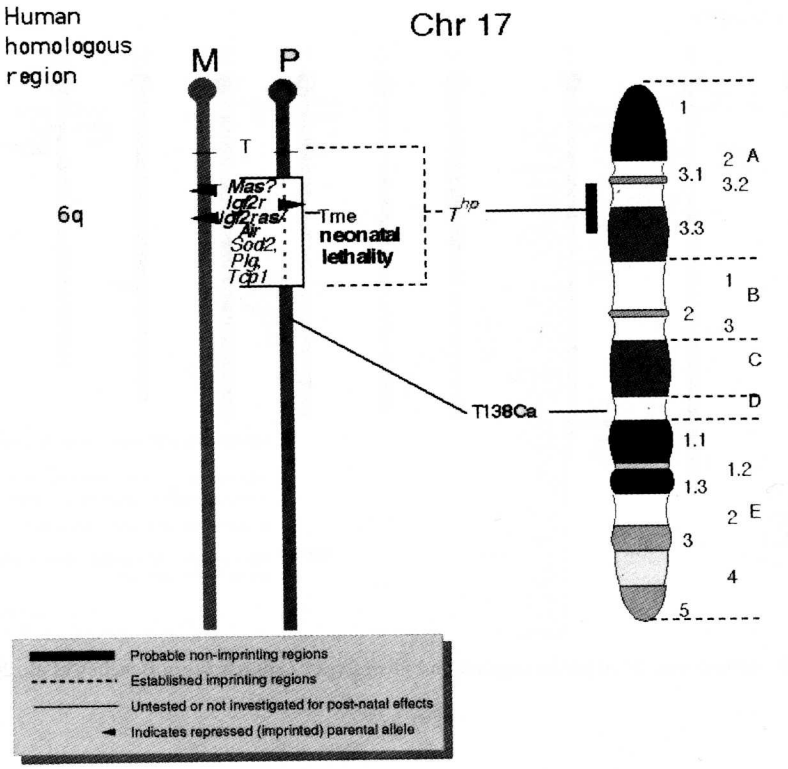

Figure 21.16 Mouse imprinting map of chromosome 17. M, maternal chromosome; P, paternal chromosome. (*Source*: From Beechey et al., 2001.)

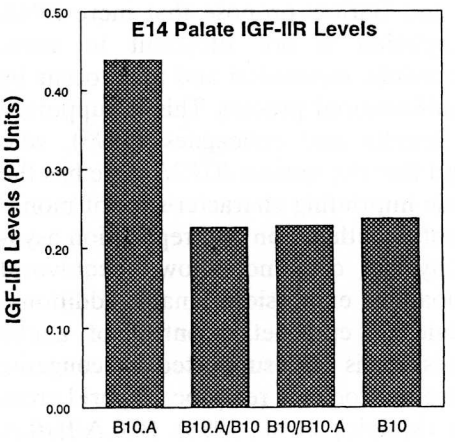

Figure 21.17 IGR-IIR mRNA levels (mean PI units) in B10 and B10.A incrosses, as well as matroclinus reciprocal hybrid crosses.

occur if the control of monoallelic expression is both biparental and B10 dominant—but how?

One possible explanation for the data in Figure 21.17 is that B10 and B10.A *Igf2r* genes have important sequence differences in the promoter regions. However, sequencing of a 900 bp region that includes the *Igf2r* promoter reveals identity in the B10–B10.A congenic pair (Fig. 21.18). Another possibility is that the critical strain differences on E14 are correlated with monoallelic *Igf2r* expression in B10 embryos and biallelic *Igf2r* expression in B10.A embryos. Thus, relaxation of methylation in B10 palates should result in IGF-IIR mRNA levels equivalent to normal

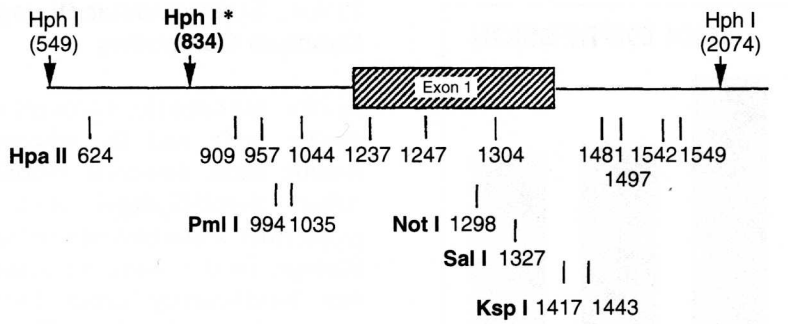

Figure 21.18 Restriction enzyme map of the *Igf2r* promotor region (not to scale). Genomic DNA and cDNA, prepared from E14 B10 and B10.A palates, were amplified by PCR and sequenced for the presence of polymorphic sites in the promotor region of the *Igf2r* genes. There is 100% identity between these congenic strains (Hoffman et al., unpublished data).

B10.A palates. Relaxation of methylation is achieved by administering the demethylating 5-Aza-2′-deoxycytidine (d-AZA) to pregnant B10 females and comparing E14 palatal IGF-IIR mRNA levels to palatal levels in untreated B10 and B10.A embryos (Melnick and Jaskoll, unpublished data). d-AZA-treated B10 E14 palates exhibit a 35% greater level of IGF-IIR transcript than untreated B10 palates and are nearly identical to untreated B10.A palate levels (Fig. 21.19). The increase in IGF-IIR transcripts in d-AZA-treated B10 and untreated B10.A is correlated with a 20+% decline in Cdk4 transcripts, and thus diminished palatal growth (Fig. 21.20).

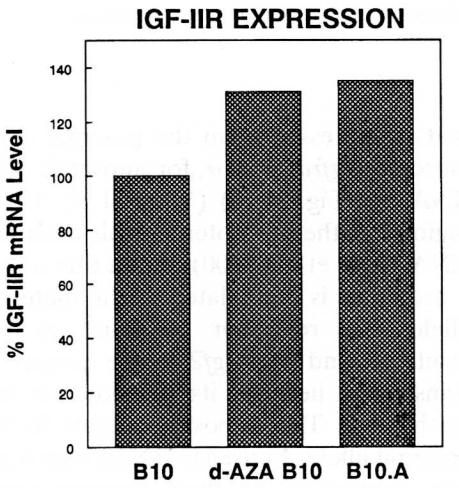

Figure 21.19 IGF-IIR gene expression in B10 and B10.A incrosses, as compared with d-AZA-induced relaxation of methylation in B10 incrosses.

These results (Figs. 21.19 and 21.20) suggest an important genetic and/or epigenetic regulation of *Igf2r* imprinting during a critical stage of palatogenesis—one that is B10 dominant. A number of *cis*-acting sequences are being defined that are also important for the control of imprinting. A *cis*-acting locus is a specific region of nucleotide sequence that affects the activity of gene(s) on that same DNA molecule; *cis*-acting loci generally do not encode proteins. Methylation differences are found in two regions of the *Igf2r* gene (Reik and Constancia, 1997). Region 1 is in the promoter, and it is methylated when the paternal allele is not expressed. Region 2 is downstream in the second intron, and it is methylated in the expressed maternal allele, suggesting that this region contains an "imprinting box" with silencer sequences that can be suppressed by DNA methylation. Indeed, it has been demonstrated that intron 2 produces an antisense

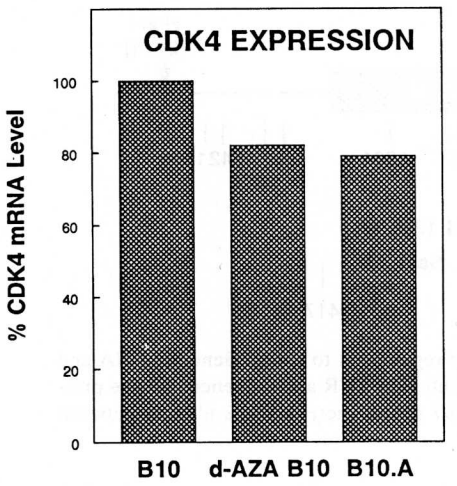

Figure 21.20 Cdk4 gene expression in B10 and B10.A incrosses, as compared with d-AZA-induced relaxation of methylation in B10 incrosses.

that is expressed from the paternal chromosome (*Igfras* or *Air*, for antisense *Igf2r* RNA; see Fig. 21.16) (Wutz et al., 1997); region 2 is the promoter for this antisense RNA (Lyle et al., 2000). When this region 2 promoter is methylated in the maternal allele, the repressor antisense is not available and the *Igf2r* gene proper is transcribed because its promoter is not methylated. The opposite obtains in the paternal allele. Antisense RNAs may regulate expression of their cognate sense mRNAs via transcriptional interference or expression competition; it is not certain if *Air* expression is a cause of *Igf2r* repression or a consequence of the repression mechanism (Lyle et al., 2000). Should it be the former, we should expect sequence differences between B10.A and B10 in the region 2 promoter for *Air*. Since a low level of paternal *Igf2r* allele expression is found in tissues that highly express the maternal allele (Lerchner and Barlow, 1997), it is reasonable to surmise that the matroclinus reciprocal hybrid cross data (Fig. 21.17) (Melnick et al., 1998) can be explained by strain differences in *Air* expression.

21.4.4 Submolecular Biology and Quantum Computing

In 1960, between the discovery of the DNA double helix and the discovery of the genetic code, two-time Nobel Laureate, Albert Szent-Györgyi, wrote a monograph titled *Introduction to a Submolecular Biology*. In this work he makes the case that biochemistry needs to follow "its parent science, chemistry, allying itself with physics and mathematics, [making] a dive into a new dimension, that of the submolecular or subatomic dimension of electrons, a dimension the happenings of which can no longer be described in the terms of classic chemistry, the rules of which are dominated by quantum, or wave, mechanics. . . . What admits no doubt in my mind is that the Creator must have known a great deal of quantum mechanics and solid state physics, and must have applied them. Certainly, he did not limit himself to the molecular level when shaping life just to make it simpler for the biochemist." Using a variety of examples, he convincingly argues that "distinguishing between structure and function, classic chemical reactions and quantum mechanics, or the sub- and supramolecular, only shows the limited nature of our approach and understanding." Were Szent-Györgyi alive today, he would still be ahead of his time. He would be heartened, though, to see that an increasing number of his colleagues view biology as a manifestation of information and computation at the cellular, molecular, and submolecular levels, and that the organism is essentially a huge, if mysterious, supercomputer parallel processing millions of bits of information (see review by Siegfried, 2000).

There is information and there is information processing. This chapter contains information coded in letters at one level and, at a higher level, in agreed-upon meanings of words, syntax, and grammar. It will stay with me unless I send these codes

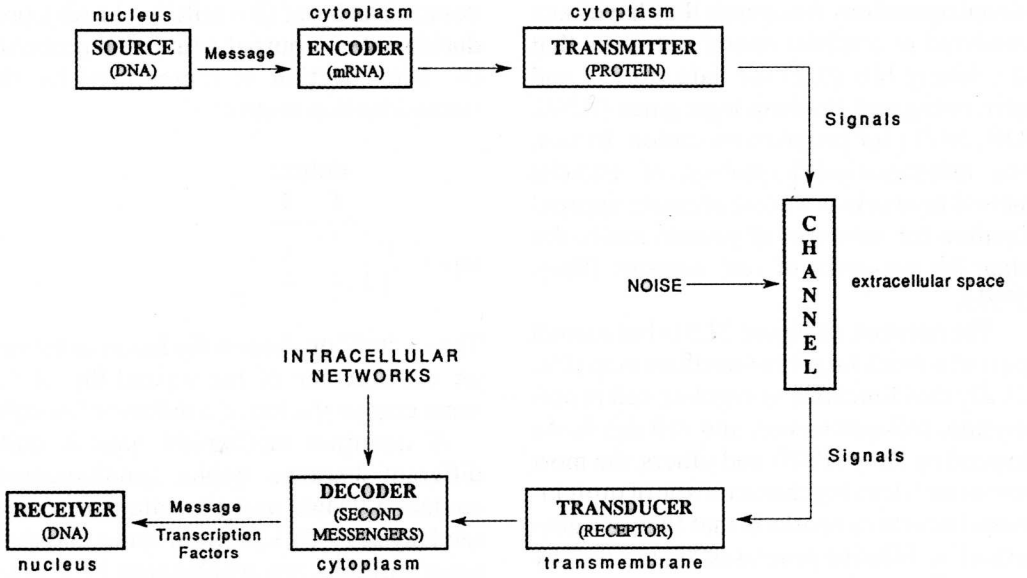

Figure 21.21 Biocybernetics: adapting information theory.

to you by some means, electronic or otherwise. Thus, there is an important difference between information coded in DNA and transfer of that information to sites elsewhere, between storing Chopin's music in your head and transferring its beauty by piano to my auditory system with some fidelity. Regarding the IGF/TGF-β epigenetic network (Fig. 21.5), we can acquire a sense of the transfer of information if we place it in the context of an adapted information theory (Fig. 21.21).

Fifty years ago, Shannon and Weaver (1949) presented a mathematical theory of how information in the form of a message or signal from a SOURCE transmitted to a RECEIVER is influenced by the CHANNEL through which that signal must pass. They concluded that in a closed system in which nothing comes in from the outside, there can never be more information presented to the RECEIVER than was initially signaled by the SOURCE. In fact, given that there is almost always "noise" in the CHANNEL, there is likely to be less information presented to the RECEIVER unless compensated for by repeated signals of the same type.

We can adapt this general scheme (Fig. 21.21) to accommodate our current knowledge (Fig. 21.5). Several features of Figure 21.21 are worth noting. First, and most important, the system is necessarily *open*. It is made so by intracellular networking, and so the RECEIVER may be presented with *more* information than was initially signaled by the SOURCE. Second, noise in this context is comprised of the vagaries of extracellular space. As such, the efficiency of the signal transmission is reduced and a general redundancy of signal is a given. Finally, the output of the RECEIVER is responsible for a number of distinct phenotypic effects (pleiotropy). Thus, proteins in epigenetic networks such as Figure 21.5 have as their primary function the transfer and processing of information; these circuits perform a variety of simple computational tasks including amplification, integration, and information storage (Bray, 1995).

Protein molecules are in priniciple able to perform a variety of logical or computa-

tional operations. As circuits they have been modeled as artificial neural networks that use binary bits (0, 1) for data storage and processing and Boolean logic gates (AND, OR, NOT) for program execution. In fact, the mathematical formalism of artificial neural networks is a more accurate approximation for networks of protein molecules than for networks of real neurons (Bray, 1995).

The network of Figure 21.5 is but a small part of a much larger connections map (Fig. 21.22) that functions to regulate cell proliferation, cell quiescence, and cell death. As argued by Bray (1995) and others, the most important defining characteristic of protein-based neural networks is that they are governed by diffusive processes. Signals pass by means of physical contact between molecules in crowded conditions, and their dispersion through the cytoplasm is limited by the random thermal motion of molecules. Even though proteins remain the fundamental units of computation in an artificial neural net, cellular computing is more accurately viewed as quantum, not Boolean (Siegfried, 2000). This is because information storage and processing is submolecular (atoms, etc.), and as such obeys quantum rather than classical laws. This has interesting implications for the functioning of the IGF/TGF-β epigenetic network (Fig. 21.5).

Since quantum mechanics appears to be an accurate formulation of nature, it governs all physical systems, including submolecular information processing. While a classical bit must be either 0 or 1, a single quantum mechanical bit, a *qubit*, simultaneously contains a 0 component and a 1 component, and n quantum mechanical bits can simultaneously represent 2^n bits at once. This "quantum parallelism" would process larger amounts of information faster than classical bit processing.

In classical Boolean logic, one simple device is a Probabilistic NOT Gate, say a gate that models a fair coin flip. In this case, we use a logic gate that completely randomizes its input (0 = tails, 1 = heads), producing a 0 or 1 output with equal probability. This function is represented by the transformation matrix:

		output 0	output 1
input	0	$\frac{1}{2}$	$\frac{1}{2}$
	1	$\frac{1}{2}$	$\frac{1}{2}$

Thus, a "tail" on the first flip has no influence on the outcome of the second flip of the same coin or the flip of a different fair coin.

A quantum mechanical gate is quite different because qubits simultaneously contain a 0 state and a 1 state (for review, see Hayes, 1995). Quantum states and their superpositions are represented by a notational device called a ket ($|\rangle$). A quantum coin flip would be represented by the following transformation matrix:

		output $\|0\rangle$	output $\|1\rangle$
input	$\|0\|\rangle$	$1/\sqrt{2}$	$-1/\sqrt{2}$
	$\|1\|\rangle$	$1/\sqrt{2}$	$1/\sqrt{2}$

The probability of each transition is the square of the corresponding value; thus, all values in the probability matrtix of this quantum coin flip are $\frac{1}{2}$ (Brassard, 1994). It would appear, then, that there is no practical difference between the classic coin-flip gate and the quantum coin-flip gate. But this is deceiving.

Probability calculations for a series of classic coin-flip gates are simply made by using the multiplication rule (e.g., probability of three heads = $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}$). However, quantum coin-flip gates in series work quite differently, and the implications for information processing in epigenetic networks (e.g., Fig. 21.5) is quite interesting. Using what we reviewed above, we can derive the following sequence:

$$\uparrow\text{TGF-}\beta 2 \rightarrow \uparrow\text{p27} \rightarrow \downarrow\text{cyclin/Cdk}$$

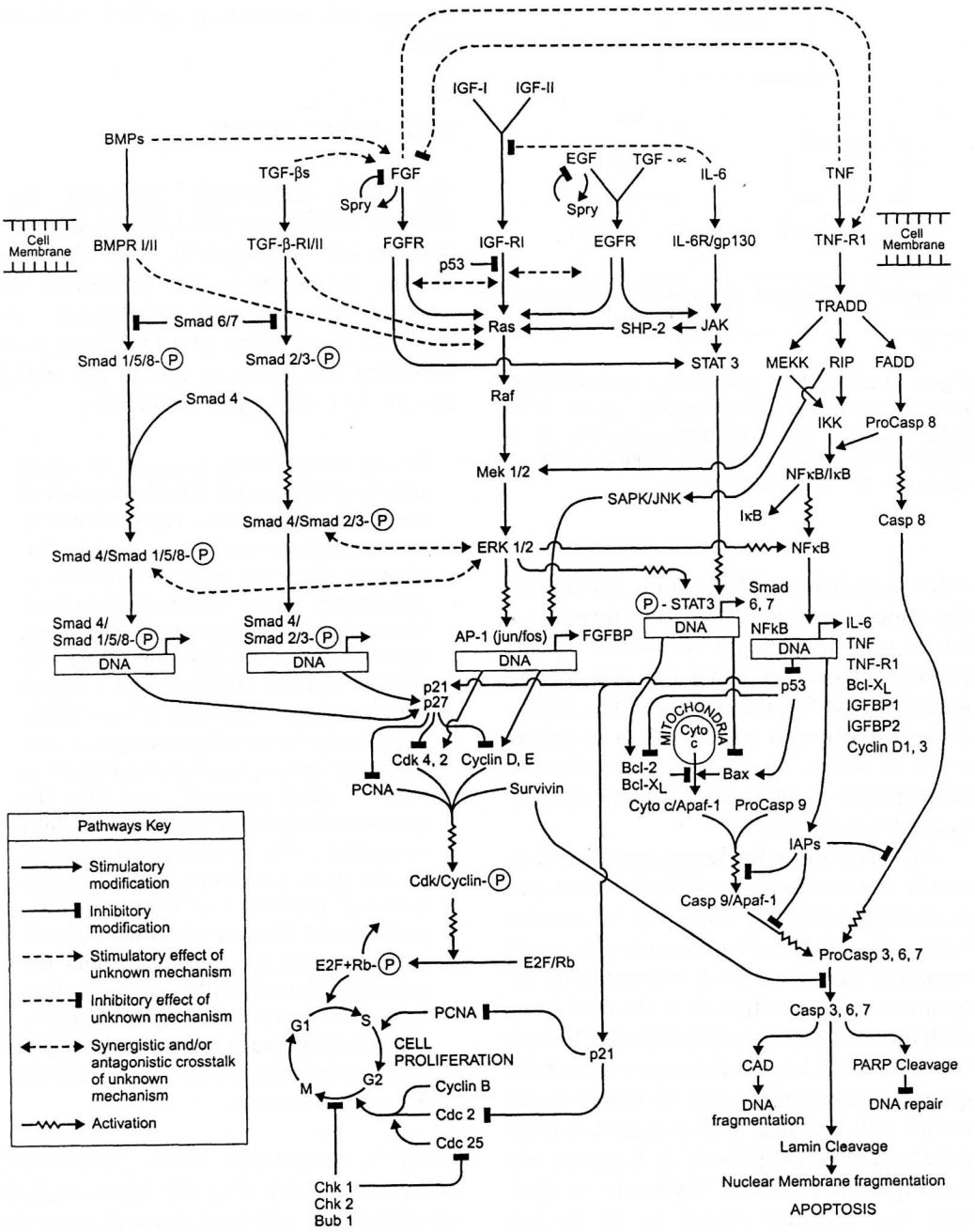

Figure 21.22 Connections map: signal transduction, cell proliferation, and apoptosis.

Thus, an increase in TGF-β2, via an increase in p27, results in a downregulation of cyclin/Cdk, and hence declining palatal mesenchymal cell proliferation. If this information were processed by a classic coin-flip probabilistic NOT gate, in response to the surmized Poissonian fluctuation of small numbers of macromole-

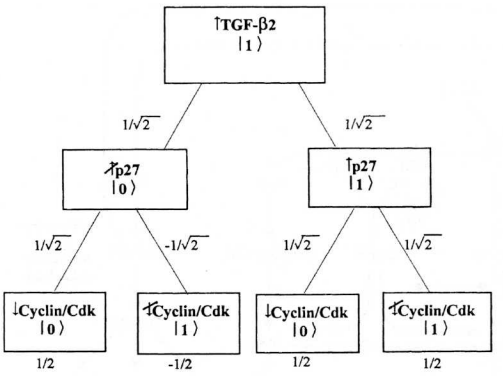

Figure 21.23 TGF-β2 upregulation, quantum NOT gates, and information processing. Note that two of the paths through a pair of quantum gates have amplitudes that interfere destructively, making downregulation of Cdk4 the certain outcome.

cules (Spudich and Koshland, 1976), then the outcomes for cyclin/Cdk (downregulation and no change) would be equally probable. However, using a quantum NOT gate (i.e., √NOT), the outcome (Fig. 21.23), downregulation, is *certain*! Thus, quantum gates in series, or the way submolecular information is processed in networks, is quite deterministic.

All this is quite intriguing and would be worth a considerable effort in computer modeling. However, since any interaction between a qubit and the macroscopic world results in decoherence, a collapsing of the quantum superposition to a classical state, such modeling has proven difficult (Taubes, 1997). In 1982, Feynman conjectured that a quantum computer would be better suited to the task and that such a quantum computer could be programed to simulate any local quantum system. Feynman's conjecture was recently shown to be correct (Lloyd, 1996). The march toward building a functioning quantum computer is steady but, unfortunately, slow (Bouwmeester et al., 2000). Nonetheless, the explosion of work in quantum information processing (e.g., Orlov et al., 2001) promises to provide valuable insight into such biologic processes as epigenetic networks (Figs. 21.5, 21.22, and 21.23).

21.5 CONCLUSION

The great Edinburgh physician, John William Ballantyne (1861–1923), capped a lifetime of investigation into abnormal human development by publishing the *Manual of Antenatal Pathology and Hygiene, The Embryo* (1904). He made the following observations about the pathogenesis and etiology of oral clefts:

> Nearly every one is prepared to admit that these fissures [clefts] are the result of arrested development, even if all are by no means at one as to the precise mechanism by which the arrest is initiated. . . . Apparently simple explanations of matters embryological and teratological have, however, on more occasions than one turned out to be fallacious, and it was so with hare-lip. . . . The facts of embryology must first be thoroughly investigated (teratological developments being utilised as hints to direct research), and then the general principles of teratogenesis must be applied to the scrutiny of the results; if this be done, I feel sure that the actual model of production of hare-lip [pathogenesis] and all other malformations will be made plain. Of course this does not mean that the cause [the etiology] which leads to the arrested developments will be discovered, although we may be in an infinitely better position to make surmises regarding its nature.

What is remarkable about Ballantyne's observations is that nearly 100 years later we cannot say a great deal more about the etiology and pathogenesis of oral clefts in humans. We can add a few cell and molecular biology terms and concepts from our work in mice, but still all we can say is that clefting results from "arrested development," and all of us who have investigated this matter for the last 25 years "are by no

means at one as to the precise mechanism" or the etiology.

This essay and its authors make no claim to knowing the etiology and pathogenesis of human cleft lip and cleft palate. The foregoing is not presented necessarily for the truth of the matter but as a humble "proof of paradigm"—a paradigm proffered by Melnick and Shields (1976) 25 years ago after thinking about the elegant work of Holliday (Holliday and Pugh, 1975), among others, on the control of gene expression during development.

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