of the bidimensional method. Milman *et al. Proc. natn. Acad. Sci. U.S.A.* **23**, 4589; 1976) detected a mutation for the enzyme hypoxanthine guanine phosphoribosyl transferase by analysis of HeLa cell extracts, Commings (*Nature* **277**, 28; 1979) has reported a polymorphism of human brain polypeptides, and genetic variants of transferrin, recognized by conventional electrophoresis were demonstrated after bidimensional analysis (Anderson *et al. Biochem. Biophys. Res. Commun.* **88**, 258; 1979).

In all cases, the variants detected by the bidimensional procedure can be attributed to mutational events leading to a change in charge on the protein molecule. Many of the variants detected by conventional electrophoresis are also attributed to amino acid substitutions leading to a change in net charge and there is good reason to think that the affected residues lie on the surface of the protein. However, this type of charge change variant is more readily detected after electrophoresis of the native protein than of the denatured polypeptides, since in denaturing conditions the frictional resistance of the molecules is greatly increased and the charge change less exposed. Nevertheless, it is interesting to note that Steinberg *et al.* (*Cell* **10**, 381; 1977) have demonstrated that progressive carbamylation of AMP dependent protein kinase leads to discrete shifts in *p*I detectable by the bidimensional procedure, which presumably represent single charge changes. However, one of the problems associated with detecting amino acid modifications by isoelectric focusing is that the efficiency of the resolution, as judged by the relative shift of *p*I, will not be the same over the whole gel, but will depend upon the position in the *p*H gradient and the proportional change in charge.

One way in which this problem could be overcome is by modification of the conditions for bidimensional electrophoresis which is commonly carried out in a broad *p*H gradient and at standard gel concentrations. So far the possibilities for varying these conditions have scarcely been explored and it seems likely that if greater flexibility is introduced then somewhat higher levels of heterozygosity might be revealed.

In contrast, the unidimensional gel electrophoresis method provides many opportunities for manipulating conditions such as *p*H, buffer ions, gel concentrations, solvent polarity, temperature and sample concentration. Effects due to the incorporation of affinity ligands in the gels and interactions with sulphydryl reagents can also be explored. And it is quite a common experience that analysis of an enzyme in a range of electrophoretic conditions leads to the identification of aditional variants and an increased estimate of heterozygosity. On the face of it the unidimensional electrophoretic methods primarily detect charge change variation, but in practice the procedure almost certainly demonstrates a range of other types of variants which arise as a result of uncharged amino acid replacements. Such variants may be revealed by differences in affinity of the allozymes for coenzymes, variable interaction with different buffer ions and altered electrophoretic mobility due to conformational distortion. □

# Growth hormone: deletions in the protein and introns in the gene

*from M. Wallis*

MODERN high resolution fractionation methods can reveal that most protein preparations are made up of several different closely-related species. In the case of pituitary growth hormone, such microheterogeneity has a multiplicity of origins, and its detailed analysis has provided a wealth of information about the biochemistry and genetics of the protein. Thus, heterogeneity at the N-terminus of bovine growth hormone is now known to be a consequence of ambiguous processing of the precursor (pregrowth hormone) while heterogeneity at residue 127 reflects an allelic variation in the population of cows from which the hormone is prepared. In the case of human growth hormone various enzymically modified forms of the protein have been described, which may reflect enzymatic processing of the hormone *in vivo*.

Perhaps the most interesting of the many growth hormone variants, however, is a form isolated from human pituitaries and characterized by U.J. Lewis and his colleagues at the Scripps Clinic, La Jolla (*J. Biol. Chem.* **253**, 2679; 1978); this they have called 20K human growth hormone, since it has a molecular weight 1000-2000 less than normal human growth hormone (22K). The 20K variant is smaller because it lacks 15 amino acid residues found in the normal form of the hormone. The precise position of this deletion has now been shown (Lewis, Bonewald & Lewis, *Biochem, Biophys. Res. Commun* **92**, 511; 1980) to include the entire sequence between positions 32 and 46 (inclusive) of the polypetide chain; otherwise 20K is identical to the normal form of human growth hormone. The variant possesses growth-promoting activity similar to that of normal human growth hormone, but lacks the insulin-like activity normally associated with the hormone. It exists in all human pituitaries, comprising about 15% of the total growth hormone content.

How is this remarkable variant formed? A clue may be provided by recent work on the structure of the growth hormone gene by H.M. Goodman and his colleagues at the University of California, San Francisco (Fiddes *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 4294; 1979). They have shown that the gene is split and contains at least three intervening sequences (introns). One of these occurs between the codons for residues 31 and 32 in the mRNA — precisely the position of the start of the deletion in 20K. The end of the deleted sequence (between residues 46 and 47) does not correspond to the position of another intervening sequence, but it is noteworthy that the last two bases of the codon for residue 46 (glutamine) are AG (Martial *et al. Science* **205**, 602; 1979), a base sequence found almost universally at the 3′ end of an intervening sequence in the primary RNA transcript of a gene (Crick *Science* **204**, 264; 1979). It is difficult to believe that this partial correspondence between a deleted sequence in the protein and an intervening sequence in the corresponding gene is a coincidence. It seems likely, therefore, either that 20K represents the product of a duplicated gene in which an intervening sequence has been lengthened to include part of a coding sequence (exon) or that the messenger RNA for 20K is produced from the same primary RNA transcript (and therefore the same gene) as normal human growth hormone, which would therefore be processed in two different ways. In the second case, a region of the primary RNA transcript that is a coding region for normal human growth hormone would be treated as part of an intervening sequence for the 20K variant, and different ways of processing a single primary RNA transcript would lead to production of two different proteins.

Whichever of the two explanations is correct, it is difficult to avoid the conclusion that the occurrence of an intervening sequence in a gene has allowed the formation of a markedly different protein variant. Gilbert (*Nature* **271**, 501; 1978) has proposed that the existence of intervening sequences may allow large changes in protein structure, and this may well be an example of such a change. It is interesting in this connection that the rate of evolution of human growth hormone is much greater than that of the non-primate hormones (Wallis *Biol. Rev.* **52**, 35; 1975). Whether the potential benefit of such large evolutionary jumps is in itself sufficient to explain the occurrence of intervening sequences, or whether other causes led to their origin and the evolutionary jumps are a consequence of their existence, remains to be seen. □

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