RECENT RESEARCH ON GENETIC REGULATION AND FUNCTION OF SOME SERUM PROTEINS.

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Intron variation

With the advent of recombinant DNA techniques it soon became clear that eukaryotic genes usually are organized in exons and introns. The polymorphic variants of serum proteins and of other immunogenetic systems which we hitherto study are, of course, ascribable to nucleotide variation in the corresponding exons. The introns are not expressed, but are cut out in messenger RNA production. Hybridization between messenger RNA and single-stranded DNA demonstrates that very substantial parts of DNA are not translated (see Chambon 1981). For a majority of genes the base pairs of the introns is twice or more the base number of the exons (see for example Efstratiadis et al 1980). In addition, the base pair composition of the introns commonly vary more than those of the exons (see Milner-White 1984). DNA hybridization techniques thus give access to a new and abundantly rich source of polymorphisms: Study the variation of allelic restriction fragments of numerous introns! As an example Krontiris and coworkers (1984) studied the allelic restriction fragments of the oncogene Harvey-ras. As is wellknown we all possess this oncogene and its DNA can easily be extracted from leukocytes. The Southern blot pattern of restriction fragments from different individuals could be systematically ordered. It is of more than passing interest that the Ha-ras alleles met with in cancer patients frequently differed from those found in normals.

The Pi system as a model system of serum protein polymorphism

As is wellknown the genetic variants of human α_1-antitrypsins within the Pi system are quite useful in forensic medicine. The protein moiety of the molecule consists of 394 amino acid residues in a known sequence. The reactive site of this inhibitor is crucially dependent upon a methionin residue at position 358, 37 residues from the C terminus. The main function of α_1-antitrypsin is not really to be an antitrypsin but to be an inhibitor for elastase from leucocytes. The S, Z and Pittsburgh variants are important from a clinical point of view. Fortunately, the Pittsburgh variant, which gives rise to intractable bleeding, is rare indeed. These variants are caused by single amino acid substitutions as shown below.

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	Abnormality	Substitution
S gene	Deficiency	264 Glu - Val
Z gene	Severe deficiency	342 Glu - Lys
Pittsburgh gene	Antitrombin	358 Met - Arg

The Pittsburgh mutation strikes exactly at the crucial Met site at position 358 and gives a complete change of the function of the molecule rendering highly active antitrombin.

The amount of antitrypsin in ZZ people is about 15 per cent of the normal level. As is well-known such persons, which are 1 in a 1000 Europeans, have an increased risk of becoming ill with pulmonary emphysema particularly if they are smokers. Straight - forward relations between structure and function are thus known to apply to α_1 antitrypsin and its variants. Normal α_1 antitrypsin is of potential therapeutic interest in the deficiency state in ZZ individuals and antitrombins are also of medical interest in common clinical syndroms associated with thrombosis (for recent reviews see Carrel et al. 1982, Jeppsson et Franzén 1982, Carrel et Travis 1985). Rosenberg et al (1984) and Courtney and his group in Strasbourg (1984) have succeded in isolating human α_1-antitrypsin cDNA clones. These clones and modifications thereof can be made to express themselves in yeast or E. coli and produce substantial amount of the desired proteins.

The methionine present in normal α_1-antitrypsin at the crucial position 358 is readily oxidizable and therefore less stable. Replacement of the reactive centre methionine by valine gives stability to oxidation and retains the elastase inhibition. This replacement and thus improval of the natural product has been achieved by Rosenberg and coworkers and by Courtney and his group. If the clinical trials will verify the expectations, this achievement may be classed as an example of instant evolution.

The function of γ-trace (=cystatin C) is found by sequencing and data bank searching

An recent example of how the function of a protein may unexpectedly be resolved is given by γ-trace, also called post-γ-globulin. This protein which is a normal constituent of body fluids was detected in 1961 by Clausen. Its amino acid sequence was elucidated in 1982 (A. Grubb et Löfberg) and was incorporated in several

sequence data banks. In 1968 a strong inhibitor of papain was isolated from hen eggwhite (Fossum et Whitaker) and the sequence of this inhibitor of a potent plant cysteine proteinase was worked out in 1984 (Brzin et al). It immediately became apparent that there is extensive homology between human γ-trace and the papain inhibitor of hen eggwhite. It was soon established that the function of γ-trace is to inhibit the human cathepsins B, H and L which are similar to papain (Barrett et al 1984) and γ-trace was accordingly renamed cystatin C.

From a medical point of view it is of interest to note that the levels of cystatin C is markedly reduced in the cerebrospinal fluid in individuals struck by hereditary cerebral haemorrhage with amyloidosis in young years. This disastrous disease is particularly common in Iceland (see A. Grubb et al 1984 and A. Grubb et Löfberg 1984). In these individuals γ-trace accumulates in the cerebral vessels.

If the function of a protein is found it is probable that important pathology will also be encountered.

Some special features of immunoglobulin (Ig) control

A special feature of Ig genetic control is allelic exclusion. The phenomenon was found already in 1961 by Mårtensson in distinguishing those human Ig allotypes which we today call G1m (f) and G3m (b). Allelic exclusion, or allelic preference if you prefer, has so far been shown to hold only for immunoglobulins although very recent data indicate that it is valid also for T cell receptor molecules (see Epstein et al 1985). An important corollary of this type of control is that Ig molecules are symmetrical. Köhler and others (see Köhler 1985) are now engaged in elucidating the control mechanism of allelic exclusion and a stochastic model now appears less probable. Immunoglobulin gene loci and the c-myc oncogene locus are without exception involved in the chromosomal translocations which regularly take place in Burkitt lymphoma (see Klein 1983). In more than 80 % of Burkitt cases the c-myc gene is translocated to the IgC heavy chain locus. The remaining cases engage c-myc and IgC light chain loci. The c-myc gene is thus invariably brought to immediate vicinity of IgC loci. As mentioned allelic exclusion is a very special feature of these loci. It is tempting to speculate that allelic exclusion might be a prerequisite for the emergency of Burkitt lymphoma. Speculation apart, I am able to inform you that Gm type frequencies in Burkitt lymphoma cases

are not significantly differing from those in normals, neither in African nor in Caucasian populations (R. Grubb et al 1985).

Observations chiefly concerning rabbit Ig allotypes have indicated that rabbits may express not only their nominal allotypes but also allotypes characteristic of other strains of rabbits. These "latent" allotypes appear irregularly and usually at a level of 1 per cent or less of the nominal allotypes. These observations have led to the concept that genes for a majority or all Ig allotypes of a species is normally present in the IgG genome of a single individual (see Kindt et Yarmush 1981). The question is now whether this concept does or does not apply to the human Ig allotypes. Most of the human Ig allotypes are markers of the constant part of the IgG molecules and none are characteristics of the hypervariable regions. They are thus generally determined by IgG H C genes. Direct studies of the human IgG H C genome shows that there are not more than 2 IgG H C genes within the haplotype of one individual (see Honjo 1983).It is diffucult to reconcile this evidence with an idea of IgH C control of possible latent allotypes in Man. An alternative explanation is therefore required. I believe that at least some of the observations may be explained within the framework of idiotypes, anti-idiotypes and internal images. Several investigators have established that in rabbits and mice there is extensive crossreactivity between the idiotypes of anti-allotypic antibodies of a particular allotypic specificity. This is true even for anti-allotypic antibodies raised in different species. It has also been shown that anti-idiotypic antibodies to antiallotypes not infrequently are similar to the allotypes themselves in inhibition and binding experiments (see Jerne et al 1982, Kazdin et Horng 1983, Cazenave et Roland 1984). The anti-idiotypies of anti-allotypes may thus mimic allotypes. These observations were made in animals but findings concerning anti-allotypic specificities in rheumatoid arthritis patients are compatible with this type of explantion (see R Grubb 1970, 1985).

CREDO

1. Polymorphism of introns is extensive and many new genetic system of restriction fragments await their detection.

2. α1-antitrypsin and its variants give a model system. Our aim should be to get comparable insight in other genetic systems, their importance in physiology and pathology.

3. The genetic control of recognition molecules of the immune system shows special features such as allelic exclusion and extensive crossreactivity between idiotypes of anti-allotypic antibodies.

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