MON 19.01.13 CLONING OF THE GONADOTROPIN HORMONE OF PACIFIC SALMON ONCORCHYNCUS KETA

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Fish gonadotropins are utilized in fish-breeding to accelerate sexual products. Hitherto the peculiarities of the life cycle of salmon did not allow ovulation by injection of hypophysis extracts by analogy with other species of fish. We have achieved ovulation of pacific salmon roe (Oncorchyncus nerka) at the fourth stage of spawning migration using high concentrations of highly purified gonadotropin preparations (Oncorchyncus keta). No indications of breeding-dress were observed during this process. Cloning of polyA$^+$RNA from Oncorchyncus keta hypophyses was done separately for males and females in the pBR322 plasmid vector of E.coli C-600 at the pst site to determine the molecular variety of proteins possessing gonadotropic activity and to establish their primary structure.

MON 19.02.01 Synthesis of oligonucleotides containing a sulphydryl group and subsequent attachment of thiol specific probes.

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Oligonucleotides containing a sulphydryl group at their 5-termini have been prepared. The sequence required is made using solid phase phosphoramidite methods. An extra round of synthesis is then performed with S-triphenylmethyl O-methoxymorpholinophosphinyl 3-mercaptopropan(1)ol. After normal deblocking this gives an oligonucleotide having an S-triphenylmethyl group attached to the 5'-phosphate via three carbon atoms. The triphenylmethyl group can be removed with $AgNO_3$ giving the free thiol. This thiol reacts with sulphydryl specific probes such as fluorescent iodoacetates. The resulting fluorescent oligonucleotides should be useful in the study of protein nucleic acid interactions and to replace ^{32}P-labelled oligonucleotide hybridisation probes.

MON 19.02.02 Overproduction of RNA molecules in vivo and in vitro

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We have been interested in obtaining large quantities of RNA molecules suitable for biochemical and structural studies. Our efforts concentrated in the overproduction, in vivo and in vitro, of RNAI, a RNA molecule of 108 nucleotides that regulates the initiation of DNA replication of the E.coli plasmid ColE1. We have fused by oligonucleotide directed mutagenesis the DNA sequence encoding RNAI to efficient promoters recognised by the SP6 and T7 polymerases and to the E.coli ribosomal RNA promoter. The relative efficiencies of the various constructions have been analysed and will be discussed. We have also observed that the efficiency of initiation of the promoters is not insensitive to the sequences that are fused downstream of the initiation site.

MON 19.02.03 A series of 5-substituted tetrazoles for phosphoramidite activation during oligonucleotide synthesis

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The linking of nucleotides during automated DNA synthesis is initiated by a proton transfer between phosphoramidites (Base = A,C,G,T) and tetrazole, followed by substitution of the reactive species by the 5'OH of the solid support-bound oligonucleotide. A series of 5-substituted tetrazoles have been synthesized and evaluated in this reaction. Certain substituents, aryl and heteroatom, confer a significant rate acceleration relative to R=H. A mechanistic rationale will be presented based on concentration and acidity effects of the substituted tetrazoles.

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R. Glockshuber, R. Mertz, J. Stadlmüller and A. Plückthun, Engineering of antibody variable domains with known structure, Bio. Chem. Hoppe-Seyler 367 Suppl., 164 (1986).

MON 19.02.04 An efficient method for oligonucleotide-directed mutagenesis

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Phosphorothioate groups can easily be introduced into DNA by substituting dNTP by dNTPαS in the polymerisation reaction. Using the (+)strand of M13mp2 as template, a mismatch primer at the multiple cloning site and replacing dCTP by dCTPαS, dCMPS is incorporated into the (-)strand, inter alia at the cleavage positions of restriction enzymes Pvu I and Nci I. These enzymes only nick such DNA in the (+)-strand. After nicking, a gap passed the mismatch is introduced with Exo III and the gap repaired. On transformation a high mutation frequency of 50 - 60 % for the Pvu I reaction, and 80 - 90 % for the Nci I reaction is observed. Thus, this approach which can presumably be extended to other restriction enzymes [Taylor et al., Nucleic Acids Research (1985) 24, 8749 and 8765] represents a rather efficient method for oligonucleotide-directed mutagenesis.

MON 19.02.05 Engineering of antibody variable domains with known structure

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The genes encoding the variable domains (V_H and V_L) of the phosphorylcholine binding antibody McPc603, whose crystal structure with and without antigen bound is known, were obtained by DNA synthesis. This antibody combining site is used by us as a model system to elucidate factors contributing to efficient hapten binding as well as the potential for stabilizing a transition state. The synthetic genes were designed for facile complete or partial replacement of hypervariable loops as well as for incorporating current knowledge about efficient expression. Bacterial expression systems are described and their effectiveness in obtaining large amounts of correctly folded protein is compared.