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Oral Communication Abstract – 2A.03

GENOMIC COMPARISON OF THE SEQUENCES CONTRIBUTING TO THE 3D STRUCTURE OF THE IgH 3' REGULATORY REGION (»30 kb) IN VERTEBRATES: CONSERVATION OF A LARGE PALINDROMIC STRUCTURE WITH A POLYMORPHISM OF THE INTERNAL ENHANCER

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Background: Three-dimensional (3D) conformation and relative arrangement of chromosomes in the nucleus has a major role in controlling gene-expression programs. Regulatory Regions as LCR perform their activity through different mechanisms by means of secondary structures and epigenetic changes. The mechanisms involved for these 3D remodeling are mediated by consensus sequences of DNA and formation of complexes DNA-protein able to localize specific genomic regions inside nuclear factories (Eskiw et al., *C S H Symp Quant Biol* 2011, volume 75: 501-506). The Immunoglobulin heavy chain (IgH) 3' Regulatory Region (3'RR), plays a crucial role in immunoglobulin production and B cell maturation. In humans, there are 2 copies of the 3'RR. Rodents have only one copy of 3'RR with an extra enhancer probably acquired by a rodent–lineage specific duplication event (D'Addabbo et al. BMC Evol Biol. 2011 Mar 15;11:71-83).

Results: To determine the evolutionary conservation and transformation of the main structures of IgH 3'RR we compared the genomic organization in vertebrates. We found that in the 8 species in which the whole genomic region was included in a fully assembled contig (mouse, rat, dog, rabbit, panda, orangutan, chimpanzee, and human), the main elements showed synteny and a highly conserved sequence. The wide 3'RR (»30 Kb in human) bears in all species a large palindromic sequence, consisting in two ~3 Kb complementary branches spaced by a ~3 Kb sequence always including the HS1.2 enhancer. The maintenance of the palindrome was despite an inter-specific divergence at sequence level. Another relevant result concerns human polymorphism of the HS1.2 enhancer, associated to immune diseases in our species. We detected a similar polymorphism in all the studied Catarrhini (a primate parvorder). The polymorphism consists of multiple copies of a 40 bp element, separated by stretches of Cytosine. The number of duplicates is up to 12 in chimpanzees, 8 in baboons, 6 in macaque, 5 in gibbons, and 4 in humans and orangutan. We confirmed specific binding of these elements to nuclear factors. The in *silico* prediction of "tetraplex" structures in 3'RR enhancers suggested to study in vitro these DNA sequences for the formation of 3D tetraplex. Our analysis includes UV spectroscopy, CD and NMR on HS1.2 polymorphic "Tetraplex" predicted region.

Conclusions: We want to remark that the palindrome is retained in evolutionary distant species. It suggests pressures for the maintenance of two self-matching regions driving a hairpin structure. The conservation of the palindromic structure and the primates polymorphic feature of HS1.2 show the relevance of these structures in directing and modulating the Ig production through the formation of three-dimensional organization. The hypothetical “tetraplex” formation and its variability for different alleles of the HS1.2 enhancer is another possible feature confirming the regulatory activity mediated by secondary structures of DNA.