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Database Searches for Binding Sites

In their Report "Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons" (7 Apr., p. 136), G. G. Loots and colleagues identify conserved noncoding sequences (CNSs) in orthologous regions of the interleukin (IL)–4/13/5 locus of several species (interleukins are growth and differentiation factors involved in the immune response). They demonstrated that germ line deletion of one region, CNS-1 between the genes *IL-4* and *IL-13*, reduced the frequency of *IL-4* gene activation.

Referring to their sequence analysis of CNS-1, Loots and colleagues say, "Binding sites for transcription factors known to regulate the expression of *IL-4* and *IL-13* were not found in CNS-1," on the basis of searches of the Transcription Factor Database (http://transfac.gbfbraunschweig.de/TRANSFAC/index.html). They included searches for GATA-3, c-Maf, STAT6, and NF-AT binding sites. Their statement has specific implications for the CNS-1 mechanism, excluding actions of known T helper cell type 2 (T_H2)–specific factors, implying a need for unknown factors.

We searched the same database for these factor binding sites in CNS-1 and obtained one conserved consensus GATA-3 binding site and two NF-AT binding sites in the published sequence. The GATA-3 site resides 68 nucleotides upstream of the CNS-1 forward primer, within the region Loots *et al.* deleted for their experiments, and is conserved between mouse and human. The NF-AT sites are 5 nucleotides upstream and 31 nucleotides downstream of the CNS-1 forward primer and are conserved between mouse and human.

Although their biological significance requires study, these sites are within regions already described to exert GATA-3–dependent augmentation of the *IL-4* promoter (*1*). GATA-3 is a T_H2-specific transcription factor (*2*) shown to exert chromatin remodeling effects on the *IL-4* and *IL-13* loci (*3*), and NF-AT family transcription factors regulate many T cell cytokine genes (*4*). These GATA-3 and NF-AT binding sites in CNS-1 may or may not be involved in its activity, but their recognition in CNS-1 is important in consideration of this study and in future work in this field.

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Response

Murphy points out an important issue concerning the use of transcription factor binding site databases, such as TRANSFAC (*1*), to characterize gene regulatory elements. Transcription factor binding sites are short (the "core sequence" is typically 4 base pairs in length) and highly degenerate; therefore, TRANSFAC searches invariably identify a greater number of false binding sites than functional binding sites. Because of this fact, we used relatively stringent search criteria to maximize the likelihood of discovering true binding sites in the conserved noncoding sequences, such as CNS-1, identified in our human-mouse sequence comparisons.

The criteria we used, as stated in reference 15 of our Report, included that the binding sites be conserved in multiple species in addition to humans and mice (rats, dogs, cows, and rabbits). This was based on the assumption that putative regulatory elements such as CNS-1 should have the same regulatory function in these mammals and, accordingly, should be a target for the same transcription factors. The second criterion used, which was not specifically outlined in our Report, was that the binding sites have a matrix similarity score (a quality rating) of ≥0.9. The default matrix similarity threshold for MatInspector (*2*), the software tool that we used to search TRANSFAC, is 0.85. Neither the GATA-3 nor the two NF-AT sites pointed out by Murphy fit both of these criteria. The 3′ NF-AT site, although conserved in all six species examined, had a matrix similarity score below the 0.9 cutoff value. The 5′ GATA-3 and NF-AT sites, although conserved in humans and mice, were not in the region amplified and sequenced in multiple species.

Using databases such as TRANSFAC to identify putative regulatory sequences that are targets for known transcription factors is currently the only computational method available for identifying such elements. Although this is clearly a valuable approach, the results of TRANSFAC searches need to be carefully scrutinized, taking into consideration the analysis of orthologous sequences in multiple species to distinguish between real and spurious binding site matches. Although our analysis of CNS-1 did not identify binding sites that met our stringent search criteria, we do agree with Murphy that individuals studying *IL-4* and *IL-13* expression should not be deterred from examining this element for binding activity of transcription factors such as GATA-3, c-Maf, STAT6, and NF-AT.

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Conservation Conundrum

P. Daszak, A. A. Cunningham, and A. D. Hyatt present a convincing argument in their Review "Emerging infectious diseases of wildlife—threats to biodiversity and human health" (*Science*'s Compass, 21 Jan., p. 443) that emerging infectious diseases (EIDs) pose a risk to wildlife, and they suggest that EIDs most often result from a change in the ecology of the pathogen or the host (or both). A situation they did not mention is that, in some cases, the protection of threatened species can increase the risk of an EID outbreak by allowing a close association between wildlife and domestic animals where one would not have naturally occurred. An important example is northern elephant seals (*Mirounga angustirostris*) (see figure at left), which were abundant in California and Baja California, Mexico, at the beginning of the 19th century before being nearly eliminated by hunting. During the population bottleneck that resulted, there may

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