

The evolutionary model proposed by Gao *et al.* is based on the idea of early, rare, genome-wide bursts of copy number change. The mechanisms underlying these bursts are not yet well characterized, but recent work on other large genomic rearrangements might be useful as a starting point for further investigations^{10,11}. It is also not clear how clonal stasis is maintained. There could be either strong negative selection against cells with additional mutations or a dilution effect drowning out small positive fitness advantages. Finally, little is known about the phenotypic effects of the copy number changes in each cell and whether mutational bursts result in gradual or non-gradual phenotypic changes.

Return of the hopeful monsters?

The conceptual reference point for non-gradual models of cancer evolution is Eldredge and Gould's seminal paper on punctuated equilibria¹², which is a Darwinian theory to explain the gaps observed in the fossil record. By calling their model 'punctuated evolution', Gao *et al.*, like the authors of previous studies^{7,8}, place themselves in this tradition and link their observations of cancer genomes to a theory of change and stability of phenotypes during species evolution. However, cancer cells are not a mating population and their genomes do not recombine, so the mechanisms underlying their evolution cannot be the same. In particular, Eldredge and Gould

proposed long periods of stasis separated by short periods of change as a direct consequence of allopatric speciation—the idea that new species can arise only from small isolated populations at the periphery of the parent species—a mechanism completely different from the mutational bursts Gao *et al.* postulate.

Mutational bursts and macromutation like chromothripsis⁸ or chromoplexy⁷ fit much better to non-Darwinian saltationist theories of evolution, which rely on drastic, sudden changes ('jumps') as the main drivers of evolution. One of the best known saltationist theories is the hopeful monsters theory proposed by Richard Goldschmidt¹³, which was ridiculed as an explanation of species evolution¹⁴ but whose idea of 'systemic mutations' might fit well to the global genomic rearrangements observed in cancer. In any case, this discussion shows that much more work needs to be done to embed cancer evolution into the wider context of evolutionary theory.

Putting genomics into tissue context

The study by Gao *et al.* is an important step toward understanding the mechanisms of molecular tumor evolution on a single-cell level. One of the next key steps will be to develop methods that preserve information on the spatial organization of tumors and phenotypic features of the cells. This information will be crucial to understand the environment in which cancer evolution happens

and what selective pressures, for example, immune response, act on cancer clones. Finally, the study by Gao *et al.* focused solely on copy number changes, but cancer evolution leaves traces in many different genome alterations. For example, a previous study⁹ profiled single-nucleotide variants and found evidence for widespread genetic heterogeneity indicating up to 15 clones in triple-negative breast cancers. To understand the impact that tumor heterogeneity has on patients, it is important to understand better how these different markers of tumor evolution are related to each other and which of them matter for diagnosis and the prediction of treatment success.

COMPETING FINANCIAL INTERESTS

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How the codfish changed its immune system

Peter Parham

A common ancestor of the modern codfish acquired a set of mutations that eliminated a major arm of the adaptive immune system—the MHC II pathway of antigen presentation to CD4⁺ T cells. Subsequent to this event, there was a radiation of these fish in which the number and diversity of MHC I genes increased in species-specific ways.

The major histocompatibility complex (MHC) I and II pathways have complementary antigen-presenting functions in adaptive immunity. MHC I molecules bind peptide antigens from intracellular pathogens to activate cytotoxic CD8⁺ T cells, whereas MHC II molecules bind peptide antigens from extracellular pathogens to activate helper CD4⁺ T cells. Until 2011, the prevailing belief in immunology was that MHC I and II molecules are both vital components of the immune system in all

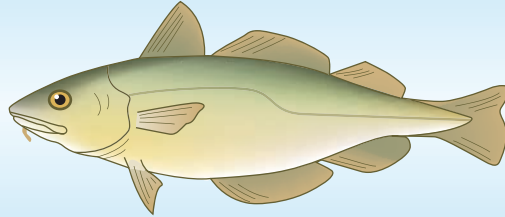
jawed vertebrates. But, in that year, completion of the genome sequence of Atlantic cod (*Gadus morhua*) showed that this robust and healthy teleost fish has no MHC II genes¹. Also absent are genes encoding proteins necessary for MHC II function¹. Notably, these include the invariant chain, a dedicated chaperone and CD4, the defining component of CD4⁺ T cells. Thus, Atlantic cod has no MHC II pathway for antigen presentation. Cod's heretical state is often unfamiliar to mainstream immunologists or is considered an exotic exception that proves the rule. Addressing the latter is a study reported in this issue in which Kjetill Jakobsen, Sissel Jentoft and colleagues, who studied the Atlantic cod genome¹, survey the condition of

MHC II pathway genes in some of the 32,000 other teleost species².

MHC II pathway loss

Malmstrøm *et al.*² sequenced 66 teleost fishes and assembled partial genomes. These fishes represent all the major lineages as well as the diversity in Gadiformes, the order of cod-like fishes. In all 27 gadiform genomes, MHC II, invariant chain and CD4 genes were in precisely the same state as in Atlantic cod. This was not the case for the 48 non-gadiform teleost genomes, 39 of which were sequenced by Malmstrøm *et al.* and 9 of which were sequenced by other investigators². This striking pattern argues that the mutations that

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Box 1 The heretical state of cod**Figure 1** Illustration of cod.

Lacking MHC II and CD4⁺ T cells, cod makes only IgM antibodies.
The amount of IgM in cod blood is ten times that of other teleost fishes.

Lacking CD4⁺ T cells, cod cannot improve antibody affinity by somatic hypermutation.
Cod does not mount an antibody response to pathogen exposure or immunization.

Cod is healthy in its natural environment.
They develop immunity to pathogens that is not antibody mediated.

Cod blood contains many phagocytic neutrophils.

Cod has an abundance of expressed MHC I genes.
Some of the MHC I molecules are predicated to mimic MHC II molecules.

Cod has made major changes to aspects of its innate immunity.
For example, cod's family of Toll-like receptors has changed the focus to be on microbial nucleic acids rather than cell surface antigens.

inactivated the MHC II pathway occurred just once, ~105 million years ago, in a common ancestor of modern gadiforms. This singularity suggests that ancient gadiforms experienced severe crisis during which fish having the MHC II pathway died out, leaving only those impaired in its functions.

Two alternatives have been proposed to explain what caused this crisis³. In one scenario, the MHC II pathway became metabolically too expensive and was abolished to cut costs. In the other scenario, use of the MHC II pathway waned, with its functions being superseded by other arms of immunity, and, once obsolete, the pathway decayed through genetic drift. However, a third possibility can be considered in which crisis resulted from epidemic infection by a pathogen exploiting the MHC II pathway to promote its own survival at the expense of its gadiform hosts. This strategy is used by HIV, which infects human CD4⁺ T cells by binding to CD4. In ancestral gadiforms, such a pathogen could have exterminated all fish with CD4, thereby selecting for fish without CD4. In these survivors, the absence of CD4 would have meant that the rest of the pathway was rendered useless and would therefore decay. Because of the interdependence of MHC II pathway components, a pathogen exploiting one component leads to loss of them all.

Although no non-gadiform teleost had the same MHC II pathway mutations as gadiforms, Malmström *et al.* found many examples of missing immune system genes². The precedent for a non-gadiform lacking the MHC II pathway is the pipefish (*Syngnathus typhle*), studied by Haase *et al.*⁴. As in cod, the genes encoding MHC II molecules, invariant chain and CD4 in pipefish are nonfunctional, but the causative mutations are different. Clearly, independent episodes of selection eliminated the MHC II pathway in the ancestors of codfish and pipefish.

MHC I pathway gains?

During the course of an immune response to infection, the major role of CD4⁺ T cells is to help B cells form antibodies of increasing avidity, specificity and capacity to recruit effector cells that eliminate the pathogen. Atlantic cod has lost this ability to refine an antibody response in real time and so exhibits unusual properties (**Fig. 1** and **Box 1**), which were first appreciated by fish immunologists during the last century⁵.

In gadiforms, absence of the MHC II pathway is accompanied by expansion of MHC I genes. The number of MHC I genes varies by species, from 2 to more than 100. Atlantic cod is at the high end of the spectrum, with many of these genes expressed⁶. This variability,

the presence of only two MHC I genes in the most primitive living gadiform and other lines of evidence concur with MHC I gene expansions having occurred after collapse of the MHC II pathway. MHC I gene expansion is just one of several compensatory responses to loss of the MHC II pathway⁷ (**Box 1**). Malmström *et al.*² found that the number of MHC I genes in a family of teleost species correlated positively with the number of species in the family. Gadiformes families were highest on both accounts. This raises the thought-provoking possibility that increasing MHC I diversity facilitates speciation, by allowing teleosts to colonize and adapt to the microbial inhabitants of a greater variety of marine environments⁸.

The MHC I genes in Atlantic cod form two groups, marked by sequence motifs in the cytoplasmic tail that determine the intracellular trafficking of the encoded molecules. One group has a conventional MHC I tyrosine motif, whereas the other has both tyrosine and dileucine motifs⁶. The latter, more typical of MHC II molecules, ensures that peptide antigens are bound in endocytic vesicles, the site of accumulation of extracellular pathogens. Consequently, an attractive but untested hypothesis is that this second group of MHC I molecules took over the functions formerly carried out by MHC II molecules. In certain circumstances, mammalian MHC I molecules can present peptides derived from extracellular pathogens that were bound in endocytic vesicles. This once heretical process is called 'cross-presentation' because it represents a cross between the MHC I and II pathways. As MHC I molecules are older and more diverse than MHC II molecules, cross-presenting MHC I molecules could have been the functional antecedent of MHC II molecules. In gadiform fish, this evolutionary progression seems to have been reversed.

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