and macrophages, which are major drivers of inflammation. Interestingly, macrophages in the synovial membrane of FAPα-cell-depleted mice had a pattern of gene expression that is characteristic of an anti-inflammatory state. This raises the question of whether disease-associated macrophages are a source of pro-inflammatory cytokines, and also whether these cells acquire an anti-inflammatory profile when numbers of FAPα-expressing cells are reduced[7]. However, this was not specifically confirmed by the authors.

To test the individual contributions of the two fibroblast populations directly, the authors isolated cells that either expressed or lacked Thy-1, and injected them into the inflamed joints of arthritic mice. Mice that received Thy-1-expressing fibroblasts developed more-severe inflammatory arthritis, but not greater bone or cartilage destruction than was the case for animals that did not receive a cellular transplant. By contrast, injection of fibroblasts lacking Thy-1 did not affect the level of inflammation, but bone erosion was greater than it was in animals that had not received a transplant. The authors concluded that the subset of Thy-1-expressing fibroblasts drove inflammation by producing cytokines, whereas the fibroblast subset lacking Thy-1 contributed to bone and cartilage destruction.

To investigate whether their findings might have relevance for human disease, the authors examined samples of cells from the synovial membrane of people with either rheumatoid arthritis or osteoarthritis — a form of arthritis characterized by joint damage but little or no inflammation[8]. They found that people with rheumatoid arthritis had a larger population of fibroblasts that express FAPα and Thy-1 than did people with osteoarthritis. Future studies should determine whether fibroblasts that express FAPα but lack Thy-1 are present in greater numbers in the synovial LL of people with rheumatoid arthritis or osteoarthritis than in the LL of healthy people, because this was not specifically examined by the authors, but is predicted by their model.

These exciting findings raise the possibility that clinical strategies might be developed for the selective depletion, targeted replacement or functional conversion of fibroblast subpopulations. Such approaches might one day provide treatment options not just for rheumatoid arthritis, but for a wide range of chronic inflammatory diseases. ■

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ANCIENT GENOMICS

Human lineages in the far north

Humans reached the Americas from northeastern Siberia during the last ice age. Genomic analyses of ancient and modern individuals reveal the history of the peoples who have populated these regions. SEE ARTICLE P.182 & LETTER P.236

ANNE C. STONE

The far northeast of Siberia was the gateway to the Americas for ancient humans, and today is home to diverse cultures whose members speak many languages. During the Late Pleistocene period (the ice age that lasted from about 126,000 to 11,700 years ago), this area of Siberia was connected to North America; the land bridge and adjacent areas formed a region known as Beringia. Hunter-gatherer populations seem to have ranged widely[1–3] across Siberia and into Beringia, sustained by megafauna such as woolly mammoths, and other animals. In this issue, Sikora *et al.*[4] (page 182) and Flegontov *et al.*[5] (page 236) examine the genetic footprints of past peoples in northeastern Siberia and northern North America, to work out their relationships to modern communities. Sikora and colleagues also examine how these peoples were affected by climate change over the past 40,000 years.

Sikora *et al.* analysed genomic data from 34 people from ancient northeastern Siberia. Two individuals were buried at Yana RHS in Russia — a 31,600-year-old archaeological site that contains the earliest human remains found in the far northeast of Siberia — and the others date from 9,800 to 600 years ago. The Yana individuals provide the only genomic data gathered so far from northeastern Siberia before the Last Glacial Maximum (LGM, about 26,500 to 19,000 years ago), although

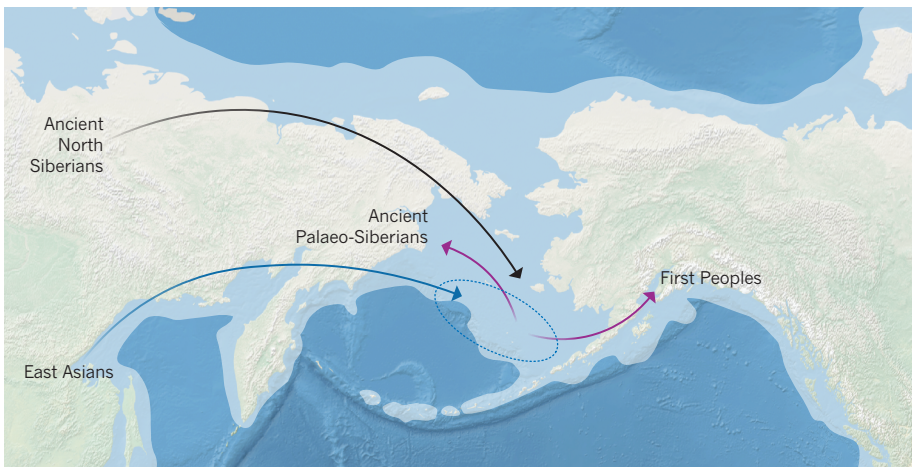

Figure 1 | Migration of ancient peoples across Beringia. During the last ice age, an area of land called Beringia connected Siberia and the Americas. Beringia is shown as the pale blue area superimposed on the modern maps of Siberia and North America. Sikora and colleagues[4] analysed DNA from the remains of ancient individuals from northeastern Siberia, and suggest that a group they call the Ancient North Siberians probably moved from Siberia to more-hospitable regions, such as southern Beringia (dotted oval), during the Last Glacial Maximum, extending from about 26,500 to 19,000 years ago. These individuals, the authors posit, were ancestral both to the first humans who inhabited the Americas (the First Peoples) and to a subsequent Siberian group (the Ancient Palaeo-Siberians). East Asians also contributed genetic ancestry to these two groups. The Ancient Palaeo-Siberian population subsequently expanded throughout Siberia, whereas the First Peoples expanded into the Americas; the two groups are estimated to have diverged about 24,000 years ago.

there is evidence of human occupation in central Siberia as early as 45,000 years ago[6].

The limited availability of genomic data from pre-LGM Eurasians has made it challenging for researchers to understand the landscape of human variation at the time. Sikora and colleagues' analyses support the idea that these populations were wide-ranging, yet structured (there were genetic differences between groups). The authors also suggest that the Yana represent a group that the team calls Ancient North Siberians (ANS), who diverged from Western Eurasians about 38,000 years ago, soon after the latter group split from East Asians.

The land bridge between Eurasia and North America existed from about 34,000 to 11,000 years ago[3,7], and it is thought that people migrated onto this bridge sometime between 30,000 and 15,000 years ago. Using palaeoclimate simulations and genetic data, Sikora *et al.* suggest that at least some ANS moved to southern Beringia during the LGM (Fig. 1), and that these individuals are ancestral both to the first people who inhabited the Americas (sometimes referred to as the First Peoples) and to another group that emerged at about the same time, whom the authors call Ancient Palaeo-Siberians. East Asians contributed 75% of their DNA to the Ancient Palaeo-Siberians, and 63% to the First Peoples, which suggests that there was some geographical separation between the latter two groups. The authors argue that these groups diverged about 24,000 years ago.

After the LGM, major environmental and cultural changes occurred on both sides of the land bridge (as they did elsewhere). In Siberia, archaeological evidence shows that a change in tool technologies occurred, coinciding with a scarcity of mammoth ivory[8]. This evidence, together with Sikora and colleagues' genetic data, indicates that population and cultural changes occurred as a result of the expansion of the Ancient Palaeo-Siberian population. The Ancient Palaeo-Siberians were then replaced by, or admixed (produced offspring) with, a group called the Neo-Siberians, between 11,000 and 4,000 years ago.

Also just after the LGM, the First Peoples began their movement southwards[9,10]. Other groups remained in the north, and it is their subsequent history that is the focus of Flegontov and co-workers' study. More specifically, the authors examine the relationships between people from several archaeologically defined cultures, including the Palaeo-Eskimos, who spread across the American Arctic from about 5,000 years ago, and the Neo-Eskimos, whose population expanded and might have replaced the Palaeo-Eskimos from about 800 years ago (Fig. 2). The researchers also study how these ancient peoples are related to modern populations who speak Eskimo-Aleut, Na-Dene and other languages.

Flegontov *et al.* examined about 1.24 million variable nucleotide sites across the genome from 48 ancient individuals and from modern Iñupiat, who live in northern Alaska. Previous research[11] has led to debate about whether the Palaeo-Eskimo admixed with other groups. Flegontov and colleagues' data demonstrate that the Palaeo-Eskimo lineage did indeed contribute to the Neo-Eskimo group, and thus its members are among the ancestors of modern Eskimo-Aleut speakers, as well as of Na-Dene-speaking peoples.

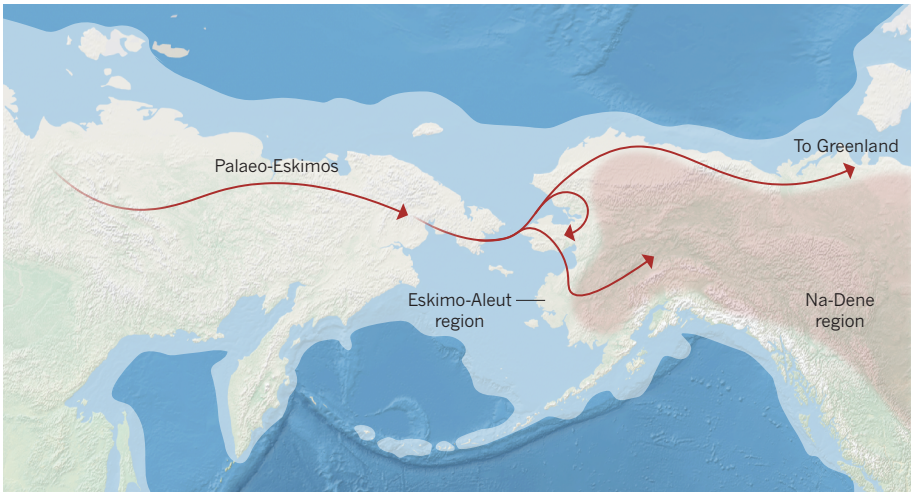

Figure 2 | Migration of ancient peoples from northeastern Siberia and across the American Arctic. Flegontov *et al.*[5] analysed the DNA of ancient and modern individuals from the American Arctic. They identify a group of ancient Siberians as the ancestors of the Palaeo-Eskimo people, who migrated from Siberia to the Americas and Greenland about 5,000 years ago. The authors find that the Palaeo-Eskimo people are among the ancestors of modern Na-Dene-speaking peoples, who inhabit the region shown in pink, and of the Eskimo-Aleut speakers, who now live in the area around the Na-Dene region.

Both of the new papers present analyses and discussions of the Palaeo-Eskimo peoples: Sikora *et al.* focus on their Siberian ancestors, whereas Flegontov *et al.* examine their relationship to subsequent populations in North America. Sikora *et al.* identify Palaeo-Eskimo individuals (including a Saqqaq individual, who lived in Greenland) as being admixtures of the Ancient Palaeo-Siberian and East Asian lineages; Flegontov *et al.* call this Siberian ancestry the Proto-Palaeo-Eskimo lineage. Both papers also describe evidence of ancient people interacting across the Bering Strait, and of migration back to Siberia. Sikora *et al.* suggest that Ancient Palaeo-Siberians contributed DNA to modern Na-Dene speakers, but (unlike Flegontov *et al.*) propose that this came from Siberian ancestors, rather than from Palaeo-Eskimos.

One limitation of the two papers is that, although some of the DNA samples analysed by the two research groups came from the same archaeological sites, it is difficult to tell whether the same individuals were sampled — a problem that can arise in studies of archaeological material. A general code of practice would be useful for this field, to encourage scientists to provide the identifiers used by the original excavators, thus enabling cross-study comparisons and validations. This would help to ensure that the destructive sampling of archaeological remains, which are non-renewable resources, is properly coordinated and minimized. The code of practice could also ensure that descendants of ancient individuals are engaged in discussions about sampling (as exemplified by Flegontov *et al.*, who note that they consulted Alaskan communities in their study).

Both studies reveal not only the complexity of the interactions that occurred within and between Siberian and northern North American populations over time, but also the impact of climate change — specifically, how the ice-age climate drove people to 'refugia' (locations where humans could survive) during the LGM, and subsequent population expansions into other regions when the ice receded or the climate improved. However, we have no human genetic data from the roughly 20,000-year period after the initial occupation of the Yana site. This is a huge gap, in archaeological terms. Further studies of Siberian and Beringian populations during this period are now needed to learn more about the genetic and cultural diversity of these groups.

More work is also needed to understand where the refugia were in northeastern Siberia, and what environmental conditions were like in these regions. In particular, what was the population structure in the Beringian refugium, and does this support the Beringian standstill hypothesis — which posits that the First Peoples became isolated during the LGM, before the southward expansion of the ice sheets[12]?

In the ongoing debate about how many 'waves' of migration led to the establishment of human populations in the Americas, the new papers could be interpreted as suggesting that there were just two: the First Peoples and the Palaeo-Eskimo peoples. If so, then how does this tally with the idea that some Amazonian populations seem to share DNA[13,14] with people who speak Austronesian languages (who live today in southeast Asia, Oceania and Madagascar)? Did the populations in the Beringian refugium also have this ancestry?

Lastly, how did environmental changes, human migrations and cultural and genetic adaptations interplay in northeastern Siberia and the far northern Americas? The two latest studies will help us to get our bearings as we work to understand the ancient humans who lived around the Bering Strait. ■

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PROTEIN ENGINEERING

Catalytic machinery of enzymes expanded

Only a few types of natural amino-acid residue are used directly by enzymes to catalyse reactions. The incorporation of an unnatural residue into an enzyme shows how the catalytic repertoire of enzymes can be enlarged. SEE LETTER P.219

ADAM NELSON

Enzymes are exceptionally powerful catalysts that recognize molecular substrates and process them in active sites. They are generally built from just 20 types of amino acid, and their catalytic machinery is typically assembled from chemical groups in the amino-acid side chains, often with extra bound metal ions or cofactors. This raises the question of whether the catalytic repertoire of enzymes could be expanded by using an extended 'alphabet' of amino acids that offers a wider range of side chains for catalysis. On page 219, Burke *et al.*[1] report the construction of an enzyme that uses an unnatural catalytic chemical group, and show that the enzyme's catalytic properties can be greatly improved using an approach called directed evolution.

The amino-acid side chains found in enzymes contain at most one chemical group, and are crucial for molecular recognition. But fewer than half of these side chains contain groups that can act as acids, bases or nucleophiles (electron-pair donors) in enzyme catalytic cycles. None of the side chains can act as electrophiles (electron-pair acceptors), which could also be useful for catalysis. The introduction of unnatural amino-acid residues that bear potentially catalytic side chains could therefore open up a wide range of new enzymatic reactions.

Conventional catalysts are a fertile source of inspiration for chemical groups that would expand the catalytic repertoire of enzymes: both small-molecule organic catalysts (organocatalysts) and transition-metal catalysts can activate substrate molecules in ways that enable a variety of reactions that are useful for organic synthesis. To enable enzymes to access this exciting reactivity, methods are required for the efficient site-specific incorporation of amino acids that bear new chemical groups. Methods for the directed evolution of the resulting modified enzymes are also required to optimize catalysis in active sites.

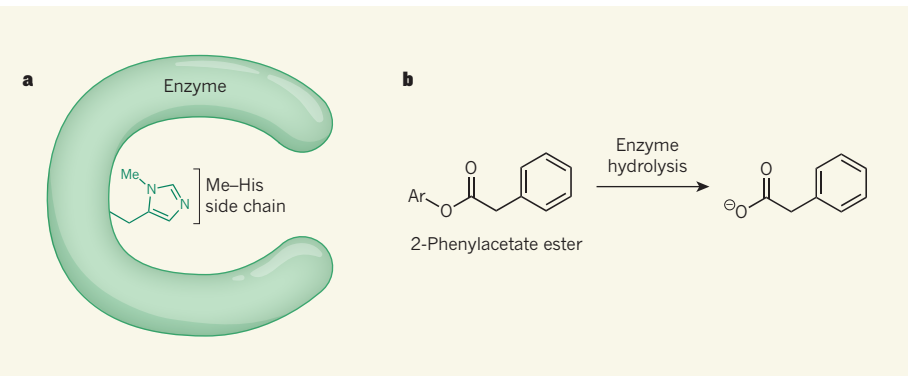

Figure 1 | An unnatural amino-acid residue remodels enzyme activity. **a**, Burke *et al.*[1] have replaced a histidine amino-acid residue in an enzyme's active site with an unnatural residue — N_δ-methylhistidine (Me-His), an analogue of histidine in which a methyl group (Me) is attached to one of the nitrogen atoms in the side chain. **b**, The authors optimized the resulting enzyme using a method called directed evolution, thereby producing an enzyme that catalyses the hydrolysis of compounds called 2-phenylacetate esters. This reaction is very different from the one that the starting enzyme had been designed to catalyse. Ar is a group that generates a fluorescent by-product on hydrolysis.

Artificial enzymes have previously been constructed by attaching transition-metal catalysts to a small molecule known as biotin, which in turn binds non-covalently with extremely high affinity to the protein streptavidin, thus anchoring the catalyst in a protein framework[2,3]. Metal catalysts have also been covalently attached to the side chains of unnatural amino-acid residues that have been incorporated into proteins using modified biological protein-synthesis machinery[4]. With both of these strategies, directed evolution was used to greatly improve the catalytic efficiency and turnover (the average number of reactions catalysed by each enzyme) of the initially produced artificial enzymes, and, in some cases, to increase the selectivity of the enzyme for a particular mirror-image isomer of the product (enantioselectivity). Artificial enzymes have thus been produced that catalyse reactions not found in nature, including silicon–carbon bond-forming reactions[4], and carbon–carbon bond-forming reactions known as cyclopropanations[4] and ring-closing metathesis reactions[2].

Burke *et al.* took a different approach. They started from an enzyme[5] (BH32) that had been computationally designed to catalyse a particular type of carbon–carbon bond-forming reaction, but which also weakly catalyses an unrelated transformation: the hydrolysis of compounds known as 2-phenylacetate esters (Fig. 1). The authors therefore decided to remodel the enzyme to make it an effective catalyst for these hydrolyses.

The researchers determined that a histidine amino-acid residue (His23) in BH32 forms an intermediate called an acyl–enzyme compound during the catalytic cycle. This intermediate is then hydrolysed to yield the product of the enzymatic reaction. However, the catalytic turnover was poor because the hydrolysis of the acyl–enzyme intermediate was slow.

To address this issue, Burke and colleagues replaced His23 with a genetically encodable, unnatural amino acid called N_δ-methylhistidine (Me-His; Fig. 1). Me-His