During its short (2 million years) but energetic lifetime and its explosive death, a first-generation star would expel all the remaining gas (more than $10^5$ solar masses) from the entire gravitationally bound clump of dark matter, thereby preventing the formation of other stars. Hence, only one atom in a thousand got incorporated into first-generation stars. More efficient star formation had to await the subsequent buildup of larger and less fragile systems with deeper potential wells that could retain gas (in effect, the first small galaxies).

The environmental impact of these very early massive stars was crucial. They emitted mainly in the ultraviolet and initiated the reionization of the intergalactic medium, a process that was not completed until the universe was 1 billion years old (*1, 2*) but must have started much earlier. They produced (and, when they died, dispersed) the first carbon, oxygen, and iron. Gas that contains even small amounts of these elements cools more efficiently. It is also more opaque, rendering radiation pressure more effective in stemming infall onto young stars. Consequently, all later stars may be of lower mass, perhaps more akin to those forming in the Orion Nebula today (see the figure).

Today, the stars modeled by Abel *et al.* are vastly outnumbered by the stars that formed later in galaxies. It will be an even greater challenge to compute the complicated internal gas dynamics and feedback in these larger systems. But perhaps we will be guided by observations. Small galaxies older than 1 billion years may be detectable with the Next Generation Space Telescope, if not by 8-m telescopes on the ground (*9*).

The very first generation of stars, although few in number, nonetheless played a crucial formative role in the emergence of all later structures. The calculations by Abel *et al.* go some way toward dispelling the mystery and uncertainty that surrounds them.


**References**

1. R. H. Becker *et al.*, *Astrophys. J.*, in press (available at http://xxx.lanl.gov/abs/astro-ph/0108097).
2. X. Fan *et al.*, in preparation (available at http://xxx.lanl.gov/abs/astro-ph/0111184).
3. T. Abel, G. L. Bryan, M. L. Norman, *Science* **295**, 93 (2002); published online 15 November 2001 (10.1126/science.1063991).
4. V. Bromm, P. S. Coppi, R. B. Larson, *Astrophys. J.* **527**, L5 (1999).
5. F. Nakamura, M. Umemura, *Astrophys. J.* **548**, 19 (2001).
6. T. Abel, G. L. Bryan, M. L. Norman, *Astrophys. J.* **540**, 39 (2000).
7. A. Heger, S. E. Woosley, *Astrophys. J.*, in press (available at http://xxx.lanl.gov/abs/astro-ph/0107037).
8. P. Madau, M. J. Rees, *Astrophys. J.* **551**, L27 (2001).
9. R. Barkana, A. Loeb, *Phys. Rep.* **349**, 125 (2001).


PERSPECTIVES: DEVELOPMENT

# Carbohydrate Recognition in Spermatogenesis

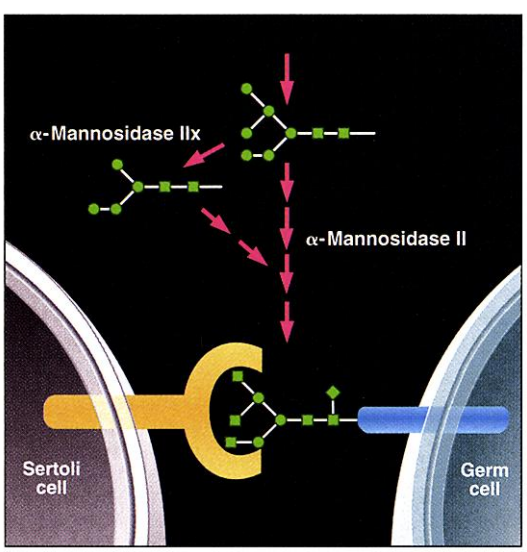
**Takashi Muramatsu**

Carbohydrates linked to proteins or lipids in the plasma membrane are involved in cellular processes as varied as embryonic development and the recruitment of white blood cells to sites of inflammation (*1–3*). On page 124 of this issue, Akama *et al.* (*4*) identify a unique carbohydrate, required for spermatogenesis in the mouse, that enables male germ cells to adhere to Sertoli cells in the testis.

Chains of sugar molecules (oligosaccharides) linked to proteins through an asparagine residue are described as N-linked. To form complex N-linked carbohydrate, a long sugar chain is transferred to the protein, is "trimmed" (*1, 5*) and then further embellished by the addition of sugars, such as *N*-acetylglucosamine (GlcNAc), galactose (Gal), fucose, and sialic acid. The enzyme α-mannosidase II is important for the final processing step in the formation of complex carbohydrate. When mice are engineered to lack the gene encoding α-mannosidase II, their red blood cells (but not other cells and tissues) lose the ability to make complex carbohydrate (*6*). The mice also develop dyserythropoietic anemia, which resembles human congenital dyserythropoietic anemia type II. The other cells and tissues of the mutant mouse retain their ability to make complex carbohydrate, probably because they produce an alternative enzyme, α-mannosidase IIx.

Akama *et al.* (*4*) report that male mice lacking the α-mannosidase IIx gene, which is predominantly expressed by male germ cells, exhibit almost complete suppression of fertility. This result complements the finding that the chemical swainsonine, which inhibits both α-mannosidase II and IIx, causes male sterility. By staining with labeled lectin, the authors discovered that a sugar structure present on male wild-type germ cells was almost undetectable on the germ cells of mice lacking α-mannosidase IIx.

So, what happened to the germ cell carbohydrate in the mutant mice? Akama *et al.* used high-performance liquid chromatographic mapping to separate enzymatically released N-linked oligosaccharides from the testes of wild-type and mutant animals (*7*). They were able to determine the structure of the N-linked oligosaccharides from the change in elution position after digestion with glycosidases. Their analysis revealed a marked decrease in oligosaccharides with GlcNAc at the terminus, but not those with galactose at the terminus, in testes from mutant mice. In an in vitro assay, the investigators then tested the effect of each oligosaccharide on the adhesion of male germ cells to Sertoli cells. They found that a unique oligosaccharide with a GlcNAc residue at




**A spoonful of sugar.** A newly identified carbohydrate on male germ cells enables them to bind to Sertoli cells in the mouse testis. The enzymes α-mannosidase II and α-mannosidase IIx process oligosaccharides to form the complex carbohydrate attached to proteins. Only α-mannosidase IIx is able to produce complex carbohydrate containing oligosaccharide chains that terminate in GlcNAc (square) and fucose (diamond), rather than mannose (circle) or galactose (not shown). It is this unique oligosaccharide that germ cells use to adhere to Sertoli cells, although the molecule on Sertoli cells (possibly a lectin) that recognizes this sugar has not yet been identified.

The author is in the Department of Biochemistry, Nagoya University School of Medicine, Nagoya 466-8550, Japan. E-mail: tmurama@med.nagoya-u.ac.jp

its terminus blocked germ cell adhesion to Sertoli cells. Furthermore, there was a 50% decrease in this oligosaccharide in testis tissue from the mutant mice. The newly identified oligosaccharide carried three exposed GlcNAc residues as well as a fucose residue attached to the core of the sugar chain (see the figure). Intriguingly, this oligosaccharide did not have an unusual linkage, but rather was produced by arrest of the biosynthetic pathway before the addition of galactose.

There are two obvious anomalies. First, although GlcNAc-terminal oligosaccharides decreased in the mutant mice, Gal-terminal oligosaccharides did not, even though the former are precursors of the latter. As the authors point out, one possibility is that α-mannosidase II may predominate in non-germ cells and process Gal-terminal oligosaccharide precursors. Alternatively, both α-mannosidase II and α-mannosidase IIx may exist in germ cells, each processing carbohydrate attached to different sets of target glycoproteins (with Gal-terminal carbohydrate presumably still processed by α-mannosidase II).

Second, the newly identified GlcNAc-terminal oligosaccharide decreased by only 50%, yet male germ cell survival was almost completely suppressed. The authors propose that this could be due to a dosage effect: Below a certain concentration of the oligosaccharide, the binding of germ cells to Sertoli cells is negligible. Another possibility is that this oligosaccharide is attached to a specific target glycoprotein whose sugar chains must be processed by α-mannosidase IIx. It is known that carbohydrates attached to glycolipids, such as seminolipid (*8*) and gangliosides (*9*), are crucial for spermatogenesis, although carbohydrate recognition was not investigated.

Germ cells must adhere to Sertoli cells to survive, and the Akama *et al.* work has now identified the key molecule involved in binding. Their finding opens up new avenues that may benefit research into the cause of human male sterility. The Akama *et al.* study illustrates the importance of cell-specific or even protein-specific glycosylation machinery for cellular recognition. An interesting subject for future research will be identification of the germ cell target glycoprotein to which the new oligosaccharide is attached. One candidate is basigin (CD147) (*10*), a highly glycosylated member of the immunoglobulin superfamily, the absence of which results in sterility in mice. Equally interesting is the nature of the molecule on Sertoli cells that recognizes the germ cell oligosaccharide. In carbohydrate-dependent cell adhesion, the specific carbohydrate sequence is usually recognized by lectins, which are classified into several groups, for example C-type lectins, galectins, and siglecs. Discovering whether a Sertoli cell lectin binds to the unique germ-cell oligosaccharide will be the next step.

**References**


1. A. Varki *et al.*, Eds., *Essentials of Glycobiology* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999).
2. J. Alper, *Science* **291**, 2338 (2001).
3. T. Muramatsu, *J. Biochem.* **127**, 171 (2000).
4. T. O. Akama *et al.*, *Science* **295**, 124 (2002).
5. A. Helenius, M. Aebi, *Science* **291**, 2364 (2001).
6. D. Chui *et al.*, *Cell* **90**, 157 (1997).
7. N. Takahashi *et al.*, *Anal. Biochem.* **226**, 139 (1995).
8. H. Fujimoto *et al.*, *J. Biol. Chem.* **275**, 22623 (2000).
9. T. Takamiya *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12147 (1998).
10. T. Igakura *et al.*, *Dev. Biol.* **194**, 152 (1998).


PERSPECTIVES: AGING

# Dietary Advice on Q

**Marc Tatar and David M. Rand**

How much you eat, not what you eat, seems to make a difference in the aging process. It is well established that reduced calorie consumption robustly extends adult life expectancy in a variety of animal models. Now, on page 120 of this issue, Larsen and Clarke show that diet quality also affects aging (*1*). In the worm *Caenorhabditis elegans*, reduced consumption of coenzyme Q (Q) dramatically extends longevity.

Among other chores, Q carries electrons and protons across the inner mitochondrial membrane to maintain the proton gradient that drives ATP synthesis (see the figure). *C. elegans* is able to synthesize the most prevalent isoform, $Q_9$, from a demethoxy-$Q_9$ ($DMQ_9$) intermediate. But worms carrying mutations in the *clock* gene (*clk-1*) lack the di-iron carboxylate enzyme required for the final hydroxylase step and so accumulate $DMQ_9$ instead of $Q_9$ (*2*, *3*). To obtain Q, *clk-1* mutants depend solely on their primary diet of bacteria, which synthesize the $Q_8$ isoform. The balance of Q isoforms in the worm is determined by both endogenous synthesis and dietary intake. Earlier work showed that worm *clk-1* mutants fail to develop or become sterile if fed *Escherichia coli* bacteria lacking Q, and that these effects could be rescued by feeding worms wild-type *E. coli* that synthesized Q (*4*). Given the importance of Q in mitochondrial electron transport, and the importance of electron transport in the production of reactive oxygen species in aging (*5*, *6*), do the quantity and quality of Q isoforms impact worm longevity?

Larsen and Clarke show that reliance upon the endogenous isoform $Q_9$ without a dietary source of $Q_8$ dramatically extends survival of the adult worm. The investigators raised worms on a diet of wild-type *E. coli* during the larval phase, and then switched them to a diet of *E. coli* lacking Q just before the worms emerged from the last larval stage as adults. Convincingly, extension of longevity was observed in both wild-type and mutant strains of *C. elegans*, as well as with mutant strains of *E. coli* in which different steps in Q biosynthesis were disrupted.

The authors uncover some important connections among *clk-1* mutants, dietary Q, and aging. The extended life-span of *clk-1* mutants lacking $Q_9$ is partly due to a general delay in larval development (*7*). The new study shows that adult life expectancy is not altered by *clk-1* mutations. Rather, young adult mutants suffer higher mortality compared with control worms, but mortality rates are reduced at later ages. In the presence of $DMQ_9$, juvenile reliance on dietary $Q_8$ may debilitate adults in an age-independent manner, which could counter any positive benefits of slower aging on mean life-span.

By shifting the dietary intake of Q between the larval and adult phases, the study reveals important age-specific characteristics. It is known that trade-offs across ages can foster the evolution of senescence (*8*). The ability to assimilate dietary Q and to synthesize endogenous Q supports growth and development, and clearly increases components of fitness expressed at young ages. This physiological capacity, however, reduces adult survival. Senescence can evolve under these conditions because the strength of natural selection is greatest upon traits expressed early in the life cycle.

Larsen and Clarke also unveil some important interactions between Q and the insulin signaling pathway that may intersect at the mitochondrion. Mutations in the insulin receptor homolog *daf-2* extend the longevity of *C. elegans* (*9*, *10*). The new study shows that a Q-less diet further extends the longevity of these *daf-2* mutants. *Daf-12* mutants, on the other hand, show slightly reduced longevity relative to controls at 25°C, and do not respond

The authors are in the Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA. E-mail: marc_tatar@brown.edu, david_rand@brown.edu