Indeed, further data from mouse models suggest that humanization of the FOXP2 protein may have altered the properties of some of the circuits in which it is expressed, perhaps those closely tied to movement sequencing and/or vocal learning (*13*).

Given these findings, it seems unlikely that *FOXP2* triggered the appearance of spoken language in a nonspeaking ancestor. It is more plausible that altered versions of this gene were able to spread through the populations in which they arose because the species was already using a communication system requiring high fidelity and high variety. If, for instance, humanized *FOXP2* confers more sophisticated control of vocal sequences, this would most benefit an animal already capable of speech. Alternatively, the spread of the relevant changes may have had nothing to do with emergence of spoken language, but may have conferred selective advantages in another domain.

FOXP2 is not the only gene associated with the human revolution (*3*). However, it illustrates that when an evolutionary mutation is identified as crucial to the human capacity for cumulative culture, this might be a consequence rather than a cause of cultural change (*8*). The smallest, most trivial new habit adopted by a hominid species could—if advantageous—have led to selection of genomic variations that sharpened that habit, be it cultural exchange, creativity, technological virtuosity, or heightened empathy.

This viewpoint is in line with recent understanding of the human revolution as a gradual but accelerating process, in which features of behaviorally modern human beings came together piecemeal in Africa over many tens of thousands of years (*6*). Recognizing the role of culture-driven gene evolution in the origins of modern humans provides a powerful reminder of how easy it is to confuse cause and effect in science.

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CELL BIOLOGY

A Sweet Send-Off

Proteins that take too long to fold are tagged with sugar to stop their failed attempts and remove them from the cell's folding compartment.

Bertrand Kleizen and Ineke Braakman

One-third of all proteins encoded by the human genome enter the cellular secretory pathway. The first compartment, the endoplasmic reticulum (ER), is specialized for protein folding, where newly synthesized polypeptides are guided by chaperones and folding enzymes to assume a final native state. Quality control is imposed when this process fails—misfolded proteins are retained in the ER and eventually degraded, thereby keeping the cell healthy and free of protein "traffic jams." For proteins that are glycosylated, triage decisions (and their timing) involve mannosidases and mannose-specific lectins that recognize an N-linked glycan (N-linked glycosylation site in which a nitrogen atom has been attached to an amino acid) on the polypeptide chain (*1*). On page 978 of this issue, Xu *et al*. (*2*) find that the folding of nonglycosylated proteins is terminated by a similar triage mechanism that surprisingly involves a mannose residue. This "O-mannosylation" (a sugar molecule is added to an oxygen atom in serine or threonine) may act as a cell's timer to stop the lingering of nonglycosylated proteins that simply take too long to fold and remove them from the secretory pathway.

Using variants of green fluorescent protein (GFP) that are not degraded in yeast, Xu *et al*. demonstrate that only slowly or improperly folding GFPs are O-mannosylated by protein *O*-mannosyltransferases (Pmt1 and Pmt2) in the ER, whereas "superfolder" GFP is not. This modification leads to loss of GFP fluorescence and increased protease sensitivity, but also increased solubility and decreased aggregation, all indicative of a less folded state. Hence, O-mannosylation not only keeps the protein soluble (required for ER exit), but prevents further folding and perhaps stimulates unfolding, and flags the protein for elimination.

In the mechanism proposed by Xu *et al*., O-mannosylation acts as a timer and stops prolonged folding of proteins, or folding that takes too many, eventually unproductive cycles. Alternatively, the Pmt1/2 *O*-mannosyltransferases may take on a more passive role and modify every consensus sequence that is exposed; these sequons may be shielded in early folding intermediates by extensive chaperone binding and by the compact structure in native, folded proteins. This leaves only late folding intermediates that are partially released from chaperones as substrates for mannosylation. Rather than folding rate, intrinsic properties of the folding protein would determine its status as a target for Pmt1/2. The rapid O-mannosylation of peptides by mammalian orthologs of Pmt1/2 (POMT1/2) is consistent with this, although the consensus mannosylation motif reported for these enzymes (*3*, *4*) is barely found in the GFP variants used by Xu *et al*. (either proline residues or structural determinants near threonines or serines). Whether active or passive, the timing mechanism may be stochastic. How many *O*-mannose residues are required for removal from the folding process is not known, nor is it clear whether O-mannosylation continues until all affinity for chaperones is lost and the fate of the protein becomes inevitable.

What makes a folding protein a substrate for O-mannosylation? The presence of serine or threonine is required (the amino acids that get modified with the sugar), but are exposed proline residues important? Proteins that sense "foldedness" in quality control usually recognize hydrophobic residues that are normally buried in the native structure, unpaired thiols, or specific *N*-glycans. However, even when such *N*-glycans are not present (*5*), or when a selection has been removed (*6*), O-mannosylation occurs. This may implicate

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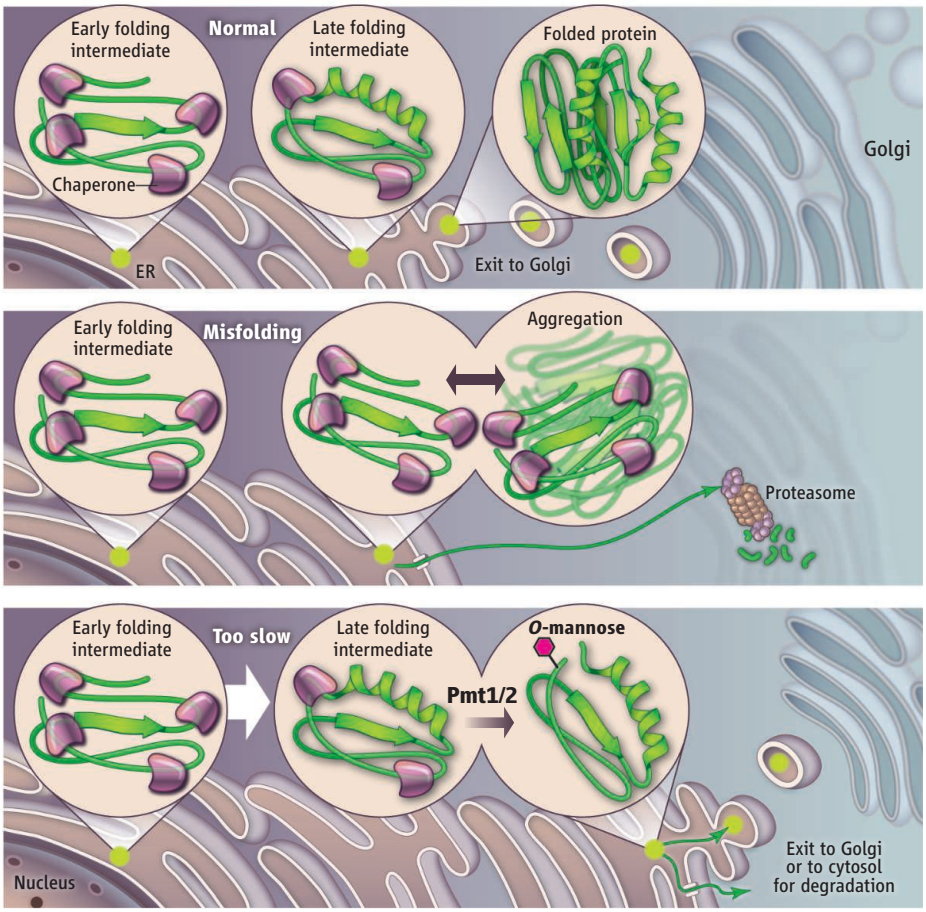

No lingering. The model shows that chaperones assist proteins during their folding in the ER. Proteins with low folding efficiency are partially released from chaperones and become O-mannosylated by Pmt1/2. This modification increases solubility, inhibits aggregation, and prevents chaperone association and further folding. Subsequent triage steps establish whether the protein will exit to the Golgi, or to the cytosol for degradation.

O-mannosylation as a triage system for glycoproteins as well.

Can Pmt1/2 (and/or its associated proteins) sense misfolding as does the mammalian ER enzyme UDP-glucose glycoprotein transferase (UGT)? UGT sends nonnative glycoproteins back to chaperones by adding a glucose molecule to their N-linked mannose chain (*7*). The Pmt1/2 proteins reside in complex with a large number of chaperones (*8*), which would allow efficient transfer of substrates that are triaged by the chaperones. This would also accommodate the passive scenario of Pmt1/2 acting on any available consensus site that presents itself in this complex.

The role of mammalian POMTs in the ER is unclear (*9*). A strong disease link exists with the Walker-Warburg syndrome, which manifests as muscular dystrophy and defects in neuronal migration. Mutations in POMT1 cause abnormal glycosylation of α-dystroglycan. In skeletal muscle, α-dystroglycan is part of a complex that links the extracellular matrix to the cytoskeleton. The POMT1 mutation disrupts this interaction (*10*); mice lacking POMT1 die before birth (*11*). The POMTs are predicted to be in the ER in mammalian cells, yet there is no evidence that they O-mannosylate proteins in that compartment. The disease symptoms and mouse phenotype suggest tissue specificity and a restricted substrate pool for the POMTs. Large-scale proteome analysis in yeast or mouse cells lacking these *O*-mannosyltransferases should demonstrate which protein substrates are affected most.

A fascinating aspect of the Walker-Warburg syndrome is that it places O-mannosylated wild-type α-dystroglycan at the cell surface, indicating that it gets through the secretory pathway and is released into the extracellular space. Also, in yeast, Pmt1/2 activity is crucial not only for degradation of O-mannosylated proteins but for exit of proteins to the Golgi complex (*8*). This is not completely surprising given that in yeast, these *O*-mannosyltransferases reside with folding factors and the ER's degradation machinery, as well as with proteins that transport secretory cargo to the Golgi, the next compartment in the secretory pathway.

These observations place O-mannosylation further upstream in the triage process, and it may constitute the first step in decision-making. In this model, O-mannosylation would stop protein folding and increase solubility (see the figure). The next step would determine either exit to the Golgi for productivity and function, or exit to the cytosol for degradation. Thus, those O-mannosylated proteins that exit to the Golgi have acquired a near-native conformation but are not very stable. As all proteins reside at the edge of their solubility and stability (*12*), O-mannosylation may help some of them escape ER quality control and move on to their functional location in or outside the cell, where partner proteins or other stabilizing conditions await them. Lectins that recognize O-mannosylated proteins would mediate either their exit from the ER or their degradation. The ER has several well-characterized mannose-recognizing proteins that are involved in the N-linked glycoprotein triage and timing process that leads to degradation. These include Htm1p and Htm2p, which are ER degradation-enhancing α-mannosidase–like proteins, and the protein Yos9p, which functions in the ER-associated protein degradation pathway. Both have been implicated in the degradation of nonglycosylated proteins as well (*1*). Perhaps this is where both degradation pathways merge—Pmt1/2 may feed the relatively low number of nonglycosylated proteins into the ER-associated protein degradation pathway used by the much more extensive clientele of N-linked glycoproteins.

The findings of Xu *et al*. may change our concept of quality control in the ER, as O-mannosylation may be the upstream triage event for all proteins and may be widespread. If so, this would have multiple consequences for human health and disease.

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A Sweet Send-Off

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