(over 175,000 amino acid sites) to over 5000 genes (over 3 million amino acid sites). Based on partitioned maximum likelihood and Bayesian analyses of their data sets, they obtained a strongly supported tree (all nodes are supported by 100% likelihood bootstrap values and a posterior probability of 1.0). All analyses supported a novel result: ants and Apoidea — the clade that includes four solitary hunting wasp families plus the bees — are sister groups. Previous studies, based on both morphology [20] and molecular data [13,14] had suggested that ants were more closely related to ectoparasitic wasps (such as Scoliidae, Tiphiidae, and Bradynobaenidae) than to the clade that includes hunting wasps and bees (Apoidea). Johnson and co-authors' analysis [10] suggests otherwise. Apoidea (including hunting wasps in the families Heterogynaeidae, Ampulicidae, Sphecidae, and Crabronidae, plus bees) and Formicidae appear to be sister groups. Likelihood-based methods for evaluating alternative hypotheses indicate that their data set can significantly reject alternative topologies, further supporting the view that their results are robust to changes in tree topology. Vespidae, the other major clade of eusocial aculeates, arises as sister to all Aculeata excluding Chrysidoidea, indicating that vespid wasps are distantly related to ants and Apoidea. While nodal support in their tree is high based on bootstrap proportions and posterior probabilities, sparse taxon sampling and a distantly related outgroup might just be leading to incorrect, but strongly supported, phylogenetic conclusions. Future studies with broader taxonomic coverage will provide additional tests of this hypothesis.

If true, the close phylogenetic affinities of ants and Apoidea would have significant implications for understanding the evolutionary origins and prerequisites for eusociality. Ants, Apoidea, and Vespidae are all groups in which females construct a nest to which prey (or pollen) is transported. Hunting wasps (and bees) are behaviorally sophisticated central-place foragers that must fabricate a nest from soil or other materials, learn landmarks associated with the location and chemical cues associated with the identity of their nest, transport prey (or pollen) over long distances, and defend the nest against predators and parasites. Nest construction, provisioning, and central-place foraging have been identified as potential prerequisites for the evolutionary origins of eusociality — a view supported by Johnson *et al.*'s [10] analysis. While the new phylgenomic results contradict previous molecular and morphological studies in terms of the affinities of ants and Apoidea, a recently described fossil [10] that has been alternatively placed in the ants and the basal Apoidea (Ampulicidae) might suggest that ants and Apoidea are more closely related than we had previously believed. This might just be a case where fossils and genomes converge on the same radical, anti-establishment view of aculeate phylogeny.

References

1. Hölldobler, B., and Wilson, E.O. (1990). The Ants (Cambridge: Harvard Univ. Press).
2. Ross, K.G., and Matthews, R.W. (1991). The Social Biology of Wasps (Ithaca and London: Comstock Publishing Associates).
3. Danforth, B.N., Cardinal, S.C., Praz, C., Almeida, E., and Michez, D. (2013). Impact of molecular data on our understanding of bee phylogeny and evolution. Annu. Rev. Entomol. *58*, 57–78.
4. Matthews, R.W. (1968). Microstigmus comes: sociality in a sphecid wasp. Science *160*, 787–788.
5. Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., and Pierce, N.E. (2006). Phylogeny of the ants: Diversification in the age of angiosperms. Science *31*, 101–104.
6. Brady, S.G., Schultz, T.R., Fisher, B.L., and Ward, P.S. (2006). Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Natl. Acad. Sci. USA *103*, 18172–18177.
7. Schwarz, M.P., Richards, M.H., and Danforth, B.N. (2006). Changing paradigms in insect social evolution: insights from halictine and allodapine bees. Annu. Rev. Entomol. *52*, 127–150.
8. Hines, H.M., Hunt, J.H., O'Connor, T.K., Gillespie, J.J., and Cameron, S.A. (2007). Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proc. Natl. Acad. Sci. USA *104*, 3295–3299.
9. Gibbs, J., Brady, S., Kanda, K., and Danforth, B.N. (2012). Phylogeny of halictine bees supports a shared origin of eusociality for Halictus and Lasioglossum (Apoidea: Anthophila: Halictidae). Mol. Phylo. Evol. *65*, 926–939.
10. Johnson, B.R., Borowiec, M.L., Chiu, J.C., Lee, E.K., Atallah, J., and Ward, P.S. (2013). Phylogenomics resolves evolutionary relationships among ants, bees, and wasps. Curr. Biol. *23*, 2058–2062.
11. Rehan, S.M., Leys, R., and Schwarz, M.P. (2012). A Mid-Cretaceous origin of sociality in xylocopine bees with only two origins of true worker castes indicates severe barriers to eusociality. PLoS ONE *7*, e34690.
12. Cardinal, S., and Danforth, B.N. (2011). The antiquity and evolutionary history of social behavior in bees. PLoS ONE *6*, e21086.
13. Pilgrim, E.M., von Dohlen, C.D., and Pitts, J.P. (2008). Molecular phylogenetics of Vespoidea indicate paraphyly of the superfamily and novel relationships of its component families and subfamilies. Zool. Scr. *37*, 539–560.
14. Debevec, A.H., Cardinal, S., and Danforth, B.N. (2012). Identifying the sister group to the bees: a molecular phylogeny of Aculeata with an emphasis on the superfamily Apoidea. Zool. Scr. *41*, 527–535.
15. Simon, S., and Hadrys, H. (2013). A comparative analysis of complete mitochondrial genomes among. Hexapoda. Mol. Phylo. Evol. *69*, 393–403.
16. Hittinger, C.T., Johnson, M., Tossberg, J.T., and Rokas, A. (2010). Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life. Proc. Natl. Acad. Sci. USA *107*, 1476–1481.
17. Lemmon, A.R., Emme, S.A., and Lemmon, E.M. (2012). Anchored hybrid enrichment for massively high-throughput phylogenomics. Syst. Biol. *61*, 727–744.
18. Hedtke, S.M., Morgan, M.J., Cannatella, D.C., and Hillis, D.M. (2013). Targeted enrichment: maximizing orthologous gene comparisons across deep evolutionary time. PLoS ONE *8*, e67908. http://dx.doi.org/10.1371/journal.pone.0067908.
19. Woodard, S.H., Fischman, B.J., Venkat, A., Hudson, M.E., Varala, K., Cameron, S.A., Clark, A.G., and Robinson, G.E. (2011). Genes involved in convergent evolution of eusociality in bees. Proc. Natl. Acad. Sci. USA *108*, 7472–7477.
20. Brothers, D.J. (1999). Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). Zool. Scr. *28*, 233–249.

Department of Entomology, Cornell University, Ithaca, NY 14853, USA.
E-mail: bnd1@cornell.edu

http://dx.doi.org/10.1016/j.cub.2013.10.026

Stress Response: Anything that Doesn't Kill You Makes You Stronger

A new study shows that DNA damage not only elicits response pathways directly related to DNA repair but also induces a response that extensively overlaps with the pathogen infection pathway and confers resistance to both oxidative stress and heat shock.

Anton Gartner[1,*] and Alper Akay[2]

Hormesis occurs when a low-level stress elicits responses that protect against subsequent exposure to severe stress. Such protection often affects a variety of stress conditions. For instance, oxidative and thermal stress

can extend lifespan by hormetic mechanisms [1]. By analogy, surviving continuous stress related to being misadvised and ill-guided as a PhD student might result in an all the more successful academic career. In a recent study, Ermolaeva *et al.* [2] report the important observation that animals subjected to a variety of stressors, such as ionising and ultraviolet radiation, and surprisingly also pathogen infection, show improved survival when later exposed to heat shock and oxidative stress.

One would conventionally assume that the DNA damage response is a cell-autonomous process; sensing of the DNA lesions leads to a transient delay in the cell cycle that allows for the efficient repair of the DNA damage [3]. If the damage cannot be fixed, cells are eliminated through a programmed cell death pathway known as apoptosis [3]. What the authors of the recent study show, using *Caenorhabditis elegans* as an organismal model system, is that DNA-damage sensing in the germ line appears to be a much more general phenomenon because they found that it initiates a stress response pathway in somatic tissues [2]. Previous studies in *C. elegans* had found no evidence for further resistance to DNA-damaging agents upon initial low doses of radiation [4]. In contrast, Ermolaeva *et al.* [2] now show that wild-type animals, which normally die very quickly at the elevated temperature of 35°C or when treated with paraquat (a reagent that generates reactive oxygen species), survived substantially longer if they were previously subjected to sub-lethal doses of either ionising or ultraviolet radiation. It is likely that the relevant DNA-damage sensing occurs in the germ line, as animals without a germ line are not resistant to heat shock upon irradiation treatment. DNA double-stand breaks are considered to be the most toxic lesions caused by ionising irradiation. The authors, undertaking an elegant set of experiments, show that DNA double-strand breaks, but not oxidative damage associated with ionising irradiation, leads to somatic stress resistance [2]. In the germ line, DNA double-strand breaks are generated naturally by the SPO-11 nuclease in order to facilitate meiotic recombination [5]. Ermolaeva *et al.* [2] find that mutant animals in which such lesions are not repaired properly and/or persist much longer [6–8] are also stress resistant, whereas *spo-11* mutants, which cannot generate DNA double-strand breaks, have a reduced level of somatic stress resistance [2].

The DAF-16 FOXO transcription factor is required to prevent premature aging and to protect against heat shock and oxidative stress [9]. DAF-16 also mediates longevity upon *C. elegans* germ line depletion [9]. In contrast to these findings, Ermolaeva *et al.* [2] show that DNA-damage-induced heat stress resistance is independent of the DAF-2/DAF-16 insulin pathway. Moreover, the 'conventional' DNA-damage checkpoint pathway, which is required for activation of DNA repair and cell-cycle arrest [10], as well as the conserved machinery mediating the apoptotic demise of *C. elegans* cells [11] are both dispensable for the observed somatic stress resistance [2].

How is it then that the DNA-damage induction in the germ line activates a stress resistance pathway in the somatic tissues? The important clue came from the analysis of the transcriptome of animals subjected to ultraviolet radiation [2] and ionising radiation [12]. Ermolaeva *et al.* [2] show that genes that are induced following both types of radiation are also induced upon infection with pathogenic bacteria. It appears that the increased expression of the secreted C-type lectin domain proteins, which are thought to have antimicrobial functions [13], is the common denominator. Indeed, the detailed analysis of the interactions between the DNA-damage-induced stress resistance and the pathogen infection response shows a clear overlap. For instance, either treating animals with ionising radiation or preconditioning them with non-pathogenic bacteria induces resistance to infection by pathogenic bacteria [2]. Furthermore, animals treated with sub-lethal doses of ionising radiation and animals infected with pathogenic bacteria are both resistant to heat stress [2]. It has been shown that MAP kinase signalling by the p38 MAP kinase PMK-1 mediates the expression of antimicrobial peptides in the gut, conferring protection against pathogens [14]. Ermolaeva *et al.* [2] show that the ERK MAP kinase MPK-1, which is activated upon ionising radiation in the germ line [15], is necessary for DNA-damage-induced stress responses. While the

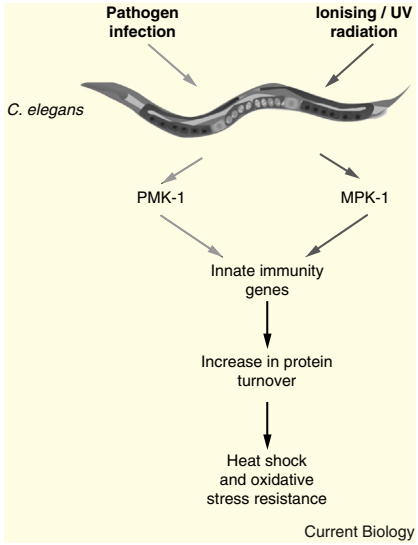

Figure 1. DNA damage and pathogen infection both activate stress resistance in somatic tissues.

Sensing of the DNA damage caused by ionising radiation and ultraviolet radiation activates the MAP kinase MPK-1 in the germ line and induces the expression of innate immunity genes in somatic tissues. Similarly, pathogen infection induces the expression of similar innate immunity genes through the activation of the p38 MAP kinase PMK-1 in the gut. In both situations, increased expression of the innate immunity genes leads to increased protein turnover and animals show resistance to heat shock and oxidative stress.

DNA-damage-induced somatic stress resistance requires a germ line, presence of a germ line is not essential for the somatic stress resistance upon pathogen infection [2]. This is in line with other studies in which *pmk-1* was shown to function in the gut upon pathogen infection [14]. Irrespective of this, both the MPK-1 and PMK-1 MAP kinase pathways activate the expression of innate immune response genes [2], the majority of which are secreted antimicrobial proteins (Figure 1).

In trying to understand the effectors of the observed stress resistance, Ermolaeva *et al.* [2] made a surprise finding. Induction of chaperones by the transcription factor heat shock factor 1 (HSF-1) is known to have a role in stress resistance and ageing [16,17]. However, even though the *hsf-1* mutants were more sensitive to heat-induced stress, *hsf-1* mutants still exhibit stress resistance to heat shock when preconditioned with irradiation [2]. Instead, the authors found that general protein stability

appears to be affected by DNA damage. Using a ubiquitin::GFP reporter system, they provide evidence that DNA damage leads to a general increase in protein turnover (Figure 1). The finding that RNAi-mediated depletion of core proteasome components blocks DNA-damage-induced heat shock resistance bolsters this hypothesis.

All in all, Ermolaeva *et al.* [2] provide strong support for the notion that 'anything that doesn't kill you makes you stronger'. What makes this study particularly appealing is the fact that phenomena that have been largely studied in tissue culture and in single-celled organisms have now been explored in intact animals. It will be interesting to study more broadly the interaction of the DNA-damage response with general stress responses and aging pathways. It will also be essential to assess the generality of these findings in other organisms. Finally, it will be important to investigate how DNA-damage signalling is emitted from the germ line to somatic tissues. Clearly there are many follow-up studies to come.

References

1. Gems, D., and Partridge, L. (2008). Stress-response hormesis and aging: "that which does not kill us makes us stronger." Cell Metab. *7*, 200–203.
2. Ermolaeva, M.A., Segref, A., Dakhovnik, A., Ou, H.-L., Schneider, J.I., Utermöhlen, O., Hoppe, T., and Schumacher, B. (2013). DNA damage in germ cells induces an innate immune response that triggers systemic stress resistance. Nature *501*, 416–420.
3. Bailly, A., and Gartner, A. (2013). Germ cell apoptosis and DNA damage responses. In Advances in Experimental Medicine and Biology, Germ Cell Development in C. elegans, T. Schedl, ed. (New York: Springer), pp. 249–276.
4. Cypser, J.R., and Johnson, T.E. (2002). Multiple stressors in Caenorhabditis elegans induce stress hormesis and extended longevity. J. Gerontol. A Biol. Sci. Med. Sci. *57*, B109–B114.
5. Dernburg, A.F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., and Villeneuve, A.M. (1998). Meiotic recombination in C. elegans initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell *94*, 387–398.
6. Colaiácovo, M.P., MacQueen, A.J., Martinez-Perez, E., McDonald, K., Adamo, A., La Volpe, A., and Villeneuve, A.M. (2003). Synaptonemal complex assembly in C. elegans is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev. Cell *5*, 463–474.
7. Alpi, A., Pasierbek, P., Gartner, A., and Loidl, J. (2003). Genetic and cytological characterization of the recombination protein RAD-51 in Caenorhabditis elegans. Chromosoma *112*, 6–16.
8. Lui, D., and Colaiácovo, M. (2013). Meiotic development in Caenorhabditis elegans. In Advances in Experimental Medicine and Biology, Germ Cell Development in C. elegans, T. Schedl, ed. (New York: Springer), pp. 133–170.
9. Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504–512.
10. Gartner, A., MacQueen, A.J., and Villeneuve, A.M. (2004). Methods for analyzing checkpoint responses in Caenorhabditis elegans. Methods Mol. Biol. *280*, 257–274.
11. Schumacher, B., Hofmann, K., Boulton, S., and Gartner, A. (2001). The C. elegans homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. Curr. Biol. *11*, 1722–1727.
12. Greiss, S., Schumacher, B., Grandien, K., Rothblatt, J., and Gartner, A. (2008). Transcriptional profiling in C. elegans suggests DNA damage dependent apoptosis as an ancient function of the p53 family. BMC Genomics *9*, 334.
13. Schulenburg, H., Hoeppner, M.P., Weiner, J. III, and Bornberg-Bauer, E. (2008). Specificity of the innate immune system and diversity of C-type lectin domain (CTLD) proteins in the nematode Caenorhabditis elegans. Immunobiology *213*, 237–250.
14. Shivers, R.P., Kooistra, T., Chu, S.W., Pagano, D.J., and Kim, D.H. (2009). Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in C. elegans. Cell Host Microbe *6*, 321–330.
15. Rutkowski, R., Dickinson, R., Stewart, G., Craig, A., Schimpl, M., Keyse, S.M., and Gartner, A. (2011). Regulation of Caenorhabditis elegans p53/CEP-1-dependent germ cell apoptosis by Ras/MAPK signaling. PLoS Genet. *7*, e1002238.
16. Hsu, A.L. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science *300*, 1142–1145.
17. Hajdu-Cronin, Y.M., Chen, W.J., and Sternberg, P.W. (2004). The L-type cyclin CYL-1 and the heat-shock-factor HSF-1 are required for heat-shock-induced protein expression in Caenorhabditis elegans. Genetics *168*, 1937–1949.

[1]University of Dundee, Centre for Gene Regulation and Expression, Dundee, Dow Street, Dundee DD1 5EH, UK.
[2]University of Cambridge, Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, UK.
*E-mail: a.gartner@dundee.ac.uk

http://dx.doi.org/10.1016/j.cub.2013.09.036

Development: Sketch for a Theory of Oct4

How is it that Oct4, a transcription factor that controls pluripotency in stem cells, also controls lineage specification? A recent study investigating common Oct4 targets in vertebrate species indicates an evolutionarily conserved role in mediating cell adhesion. This finding may help decipher Oct4's versatility in governing stem cell behaviors.

Ryan T. Wagner[1] and Thomas P. Zwaka[1,2,*]

Stem cells are defined by two different qualities: they can either divide endlessly, maintaining their pluripotent state, or they can differentiate into myriad specific cell types. This dual potential is mirrored in the behavior of the three canonical transcription factors — Oct4, Sox2, and Nanog — that both govern stem cell self-renewal and determine cell fate decisions [1]. For example, Oct4 is induced during TFG-β signaling to promote the specification of cardiac mesoderm through cooperation with canonical Wnt signaling [2,3]. How can one factor promote both the maintenance of stem cell identity and determine specific cell fates? More puzzling still, these transcription factors bind to thousands of targets in the genome, and in fact bind to many of the same targets [4]. Genomic analysis has been instrumental in detailing unique gene regulatory networks and epigenetic states in pluripotent cells [1,5], but the cellular milieu in which these factors are expressed likely imparts context-dependent activity that is less accessible to high-throughput sequencing technology. To provide a complementary perspective to the question of how developmentally relevant transcription factors exert cell fate control, Livigni *et al.* [6] took an evolutionary approach, as reported in this issue of *Current Biology*, by studying Oct4 targets conserved across three vertebrate species.

Oct4, a homeodomain transcription factor of the POU family, has been conserved to some degree throughout vertebrate development. POUV factors expressed in *Xenopus* and *Axolotl* not