doi:10.1038/nature17398

Supplementary Discussion

Possible scenarios for chondrocyte and cartilage origins.

Our analysis of cartilage formation revealed deep conservation between two distantly related protostomes and vertebrates, supporting the hypothesis that a common genetic program for cartilage development exists across the Bilateria (Fig. 4c). These findings indicate that, despite the apparent convergent evolution of cartilage tissue in vertebrates, arthropods, and molluscs, there is a deep homology[1] of the genetic program for cartilage development.

The majority of protostome and deuterostome lineages lack cartilage (or cartilage-like) endoskeletal tissues. The phylogenetic distribution of cartilage among the main three branches of Bilateria shows a lack of historical continuity of this tissue type. This distribution suggests that cartilage evolved more than once within Bilateria, although the parent cell type may have persisted in the absence of differentiated cartilage. Alternatively, while less parsimonious, it is formally possible that cartilage could have evolved in the last common ancestor of Bilateria, but was then lost multiple times (again, loss of cartilaginous tissue does not necessarily imply loss of the parent cell type or the gene regulatory network (GRN)). In light of the data presented here, we see three possible scenarios for the evolution of cartilage (only scenarios 2 and 3 are shown in Fig. 4c):

1) Evolution of cartilage/chondrocyte predates the divergence of bilaterian lineages: In this scenario, the ancient genetic circuit that we refer to as the core chondrogenic GRN is causally correlated with the origin of chondrocytes. Therefore, the chondrocyte is a homologous cell type in cartilaginous endoskeletal tissues of Bilateria. Accordingly,

similarities in the structure of cartilage ECM and in the regulation of chondrogenesis are due to common descent. A significant implication of this model is that bilaterian lineages that lack cartilage must have lost this tissue type during their evolutionary history. This hypothesis predicts that homology of the core chondrogenic GRN in Bilateria underlies homology of cartilage and the chondrocyte, as a tissue and cell type, respectively. We find this hypothesis to be the least likely of the 3 proposed here.

2) Independent origins of cartilage/chondrocyte from a homologous cell type: An alternative to the above interpretation is that chondrocytes evolved independently and in parallel, but from a homologous progenitor cell type. Therefore cartilage (and cartilage-like) tissues in different bilaterian lineages could be sister tissue types that evolved by diversification of a common ancestral progenitor. Conservation of a homologous GRN does not necessarily imply homology at higher levels of biological organization, such as the cell type (chondrocyte) or tissue type (cartilage). It is plausible that connective tissue made from other cell types could be the source of cartilage-forming cells. For example, chondrocytes could have evolved independently from homologous (yet non-cartilaginous) fibroblast-like cells in multiple lineages of Bilateria. In this scenario, parallel evolution of chondrocytes from a fibroblast-like parent cell type involved redeployment of core GRN multiple times within Bilateria. This putative fibroblast-like cell type could still be present in bilaterian lineages that lack cartilage-like tissues. This model proposes non-homology of cartilage as a tissue type due to the lack of historical continuity of the chondrocyte as a cell type across Bilaterian. However, a homologous putative fibroblast-like parent cell type would serve as a recurrent source for convergent evolution of chondrocytes across Bilateria. A similar phenomenon could underlie other cases of convergent evolution, such as the independent origin of

specialized placental tissues and viviparity in mammals and in some reptile lineages (i.e., placental tissues may have arisen independently from homologous extra-embryonic tissues, specifically the chorioallantois)[2].

3) Independent origins of cartilage/chondrocyte from non-homologous cell types: A third scenario for the origin of bilaterian chondrocytes involves the independent recruitment of a homologous GRN by non-homologous cell types. By contrast to scenario 2 (above), in which the GRN is recurrently activated to the same cell type, this third scenario proposes that the tissue/cell type where the GRN is activated could have been potentially different every time cartilage evolved. Accordingly, the independent evolution of cartilage implies non-homology at high levels of organization (structure and cell types) but deep homology of the chondrogenesis GRN within Bilateria. A frequently cited example of this process is the independent evolution of animal appendages, which show no evidence of homology at higher (structural) levels of organization, yet they display deep homology of the GRN that controls appendage development[1]. This study provides functional molecular evidence for deep homology of the GRN controlling chondrogenesis.

Supplementary references

1 Shubin, N., Tabin, C. & Carroll, S. Deep homology and the origins of evolutionary novelty. *Nature* **457**, 818-823, doi:10.1038/nature07891 (2009).

2 Blackburn, D. G. Chorioallantoic placentation in squamate reptiles: Structure, function, development, and evolution. *Journal of Experimental Zoology* **266**, 414-430, doi:10.1002/jez.1402660508 (1993).

Supplementary Tables

Table S1. Sequence identifiers for annotated reference sequences (Ref-seq) and non-annotated expressed sequence tags (EST) in NCBI used for the collagen phylogenetic analysis.

Species name	Genbank
Acyrthosiphon pisum	XM_001942728
Alvinella pompejana	AAC35289
Anopheles gambiae	NT_078267
Apis mellifera	XM_391942, XM_393523
Aplysia californica	EB249446, GD229044
Arenicola marina	AAC47545
Branchiostoma floridae	ABG36939
Ciona intestinalis	NP_001029004, XP_004226827
Cochliomyia hominivorax	FG294109
Daphnia pulex	FE335735
Epiperipatus sp.	AM499446
Euprymna scolopes	DW282906, DW252688
Haematobia irritans	FD463616
Haliotis discus	BAA75668, BAA75669
Helobdella robusta	EY380843, EY365668
Hirudo medicinalis	FP591665
Hirudo medicinalis	EY479974
Homo sapiens	AAB69977, AAH86874, BAD92412, BAD92923
Ixodes scapularis	EL516416
Locusta migratoria	CO856142

Lottia gigantea	FC620367, FC564501
Petrolisthes cinctipes	FE776091
Tribolium castaneum	XM_966372

Table S2. Sequence identifiers for annotated reference sequences (Ref-seq) and non-annotated expressed sequence tags (EST) in NCBI used for the phylogenetic analysis of Sox genes.

Species name	Genbank
Metaseiulus occidentalis	XM_003746176, XM_003741168, XP_003746224
Mus musculus	XM_006529276, NP_035578, NP_035576, NP_033260
Xenopus laevis	NM_001087769
Drosophila melanogaster	NM_166792, NP_651839, NP_523739, NP_476894, NP_001014695
Strongylocentrotus purpuratus	NM_214472, XP_786809, XP_791983, XP_798084
Nasonia vitripennis	XM_001603093, XP_001604932, XP_001603143
Ixodes scapularis	XM_002415871, XP_002404127, XP_002415916
Crassostrea gigas	BQ426397
Apis mellifera	XP_001122996, XP_006562841, XP_006570327
Anopheles gambiae	XP_560125

Table S4. *S. officinalis* embryos used for each drug treatment and for DMSO controls. A total of 72 embryos were used to test the effects of small molecule inhibitors on chondrogenesis. Treatment groups were analyzed by *ColA* and *Hh* in situ hybridizations, PCNA/ βcatenin immunofluorescence and/or Masson's trichrome histological staining.

	Total *n*	Masson's	*ColA*	*βcatenin*	*Hh*	PCNA/βcatenin	Death
DMSO	21	5	4	3	3	5	1
Alsterpaullone	19	4	4	3	3	4	1
SANT-1	15	4	4	3	-	4	0
Cyclopamine	16	4	4	3	-	4	1
BIO	5	4	-	-	-	-	1
IWR-1	4	4	-	-	-	-	0
PNU	4	4	-	-	-	-	0
Total	84	29	16	12	6	17	3