Title: Molecular anatomy of the pre-primitive-streak chick embryo

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Supplementary figures

Figure S1. Progression of hypoblast in the prestreak stage embryos. Sagittal sections on anteroposterior axis stained with PKC ζ and DAPI shows gradual progression and length of hypoblast sheet.



Figure S2. PAR3 and PKC ζ don't show regional difference in expression. (A) No difference in expression was seen for both PAR3 and PKC ζ between anterior and posterior sides in sections of EGK XI-XIII. (B) There is also no difference between the AP and the AO. Scale bars, 100 μ m.



Figure S3. RNAseq results of different regions of prestreak chick embryos at EGK.XII-III. (A) A total of 12 samples was collected for RNAseq (table). The precise location for sampling is shown with dorsal and sagittal section views. Arrows on the right side denote orientation of an embryo: A, anterior; P, posterior; L, left; R, right; D, dorsal; V, ventral. area opaca, AO; marginal zone, MZ; area pellucida, AP; Koller's sickle, KS; germ wall, GW. (B) Hierarchical clustering heat map of RNA-seq data. Color bar indicates the level of gene expression: red, high expression; blue, low expression. area opaca, AO; marginal zone, MZ; area pellucida, AP; Koller's sickle, AO; marginal zone, MZ; area pellucida, AP; Koller's sickle, KS; germ wall, GW. Lowercase a and p indicate anterior and posterior, respectively (C) Principal components analysis of RNA-seq. Colored circles indicate distinguished embryonic regions: red, AO; yellow, MZ; green, AP; blue, lower layers.





Figure S4. Expression of the other listed genes in Supplementary table S3-8, which are not suitable for a marker due to low expression. Expression pattern of genes revealed by in situ hybridization in whole mount at three stages: EGK X-XI, EGK XII-XIII, EGK XIV-HH 2. Whole embryos shown anterior side-up. Note that *CDC42EP2* and *VLDLR* have undetectable level of expression, while *MIXL1* is not available for its RNA-probe.



Figure S5. Differential cell polarization of the pMZ compared to the pAP. Double staining of ASTL mRNA (A) for labeling the MZ and RAC1 protein (B-D) for showing cell shape reveals distinct cell polarization in the pMZ. The boxed regions in (A) and (B) (the upper for the pAP and the lower for the pMZ) are enlarged in (C) and (D), respectively. White arrows indicate the KS. Scale bar, 100 μ m



Figure S6. Expression pattern of *NODAL* and *cVG1* in the prestreak stage embryos. mRNA expression patterns of *NODAL* (A) and *cVG1* (B) are shown by wholemount in situ hybridization and by paraffin sectioned images (A', A", B', and B") at three different stages; EGK.X-XI, EGK.XII-XIII, EGK.XIV-HH.2. Embryonic regions with distinct expression are denoted on the panels of EGK.XII-XIII. Dotted lines indicate the position of sectioning. Orientation: embryos, anterior side-up; sections, posterior side-right. Scale bars, 100 µm.



Supplementary table 1. Sampling details for RNAseq

ID samples	Description	No. of embryos	ng/µL	RIN	Elution volume
AO	area opaca	12	104	7.4	30
MZ	marginal zone	16	111	7.5	30
AP	area pellucida	12	226	6.7	30
aAO	anterior AO	20	33.6	7.1	30
aMZ	anterior MZ	20	18.5	7	30
aAP	anterior AP	20	29.2	8.1	30
рАО	posterior AO	20	30.1	7.5	30
pMZ	posterior MZ	20	23.2	7.1	30
рАР	posterior AP	20	50.5	5.8	30
KS	Koller's sickle	20	40.4	6.1	30
Hypoblast	hypoblast	20	16.4	7.7	30
GW	germ wall	20	13.1	8.2	30

Supplementary table 2. Number of cells (embryos) used in quantification of average aspect ratio in Fig. 2

For Fig. 2 D-E			
	X-XI	ХІІ	XIII
AP	2119 (13)	614 (3)	768 (3)
AO	99 (6)	32 (2)	68 (2)
For Fig. 2 J-K			
	ant		pos
Х	983 (5)		863 (5)
XIII	533 (3)		616 (2)

Supplementary table 3. List of MZ enriched genes

	FPKM				Fold change*					
Gene name	MZ	aMZ	pMZ	MZ/AO	MZ/AP	aMZ/aAO	aMZ/aAP*	pMZ/pAO	pMZ/pAP	
ASTL	293.324	152.801	292.207	4.683	2.105	3.780	0.498	2.394	1.584	
ARL4C	212.265	167.121	302.080	1.640	1.485	1.665	1.397	2.236	1.627	
TBX6	90.271	24.811	189.033	3.302	1.649	2.375	0.865	3.571	2.361	
CDC42EP4	38.113	27.147	38.554	2.129	2.197	1.612	1.527	1.472	1.446	
MIXL1	27.252	20.281	114.762	6.697	1.437	8.123	4.802	14.292	2.839	

*Threshold; Fold change > 1.4 except comparison between aMZ and aAP

Supplementary table 4. List of AP enriched genes

	FPKM	(M Fold change*									
Gene name	АР	AP/AO	AP/MZ	aAP/aAO	aAP/aMZ	pAP/pAO	pAP/pMZ				
RNH1	306.396	6.534	3.358	3.933	1.938	7.387	3.406				
MAFA	104.023	7.117	4.205	4.463	1.604	9.303	4.769				
ADMP	82.4662	302.806	8.135	22.937	1.936	203.671	5.987				
SFRP1	70.1655	21.325	5.798	20.552	4.867	19.080	4.826				
NKAIN4	56.2542	11.457	4.537	11.428	2.364	12.894	3.262				
LOC769139 (feather keratin 1-like)	52.6645	10.455	4.004	7.405	2.261	16.797	8.498				
LFNG	46.5435	13.247	4.076	11.380	2.892	11.633	3.867				

* Threshold; Fold change > 3 except comparison between aAP and aMZ

Supplementary table 5. List of AO enriched genes

		FPK	(M		Fold change*					
Gene name	AO	aAO	рАО	GW	AO/MZ	AO/AP	aAO/aMZ	aAO/aAP	pAO/pMZ	pAO/pAP
DLL1	102.039	110.579	92.892	279.471	8.561	490.146	13.132	107.351	11.827	792.596
RAB20	71.599	77.869	80.821	161.129	3.382	3.956	3.141	4.393	3.175	3.820
ENSGALG00000042427 (CR524387/ChEST243a9)	55.052	66.915	60.638	74.3593	3.981	3.045	3.489	4.559	3.661	3.065
LRIG1	51.465	42.642	42.214	86.6624	4.774	18.635	5.799	6.537	3.538	30.218
DOC2B	34.220	38.193	34.828	90.0425	3.825	8.116	5.209	7.594	4.031	10.236
VLDLR	32.377	41.066	39.651	52.602	3.303	7.781	3.622	5.756	7.758	9.667
HOXA2	31.400	28.994	22.881	40.834	4.177	53.797	4.757	29.407	4.614	74.277

Supplementary table 6. List of GW enriched genes

Cono namo	FPKM	Fold change*										
Gene name_	GW	GW/AO	GW/MZ	GW/AP	GW/aAO	GW/aMZ	GW/aAP	GW/pAO	GW/pMZ	GW/pAP	GW/KS	GW/Hypoblast
DKK1	804.421	2.775	10.525	62.838	2.573	23.665	53.478	2.685	4.854	34.622	2.510	2.387
DLL1	279.471	2.739	23.448	1342.442	2.527	33.188	271.313	3.009	35.583	2384.565	48.639	71.977
WNT8A (cWNT8C)	200.451	2.695	10.677	385.635	2.546	17.044	265.939	3.168	6.086	258.778	4.356	38.646
DOC2B	90.043	2.631	10.064	21.354	2.358	12.281	17.903	2.585	10.421	26.464	11.954	5.695
HEY1	87.293	2.399	5.789	14.361	2.262	6.183	10.297	2.397	6.358	12.043	6.084	4.502
ANGPTL4	50.699	2.359	3.479	2.314	3.138	5.444	4.212	2.502	5.139	3.287	3.075	3.322

Supplementary table 7. List of KS enriched genes

Cononomo	FPKM	Fold change*									
Gene name	KS	KS/AO	KS/MZ	KS/AP	KS/aAO	KS/aMZ	KS/aAP	KS/pAO	KS/pMZ	KS/pAP	KS/Hypoblasts
PITX2	143.614	7.914	5.858	11.839	13.224	13.439	14.022	2.376	2.242	4.043	2.084
CHRD	117.763	127.637	13.780	7.772	266.686	10.355	50.639	68.254	2.507	2.796	2.566
CSTA	48.238	4.208	9.835	9.067	4.817	13.105	20.361	3.169	4.191	6.242	2.900
SPIK5	30.460	13.311	27.318	153.651	24.488	62.184	231.864	4.488	3.844	109.172	13.139
IGFBP1	15.121	32.652	24.379	68.799	17.978	48.726	16.368	13.760	12.525	33.547	2.594

Supplementary table 8. List of hypoblast enriched genes

Gene	FPKM					F	old change	*				
name	name Hypoblast		Hypoblast /MZ	Hypoblast /AP	Hypoblast /aAO	Hypoblast /aMZ	Hypoblast /aAP	Hypoblast /pAO	Hypoblast /pMZ	Hypoblast /pAP	Hypoblast /KS	Hypoblast /GW
HHEX	865.737	288.950	318.273	40.845	335.791	245.972	80.792	266.262	140.450	35.771	13.753	65.590
LOC417741	531.92	166.950	199.559	34.358	180.023	76.222	39.268	185.403	171.954	50.532	19.688	44.644
LHX1	150.074	468.019	283.602	46.174	967.283	283.419	101.614	920.394	70.080	49.758	10.744	70.398
ST6GAL1	107.216	103.505	127.525	14.560	105.666	133.659	48.782	169.485	76.107	27.838	16.188	42.604
CHST1	89.599	102.995	68.779	33.584	105.263	70.757	51.911	162.761	86.868	40.455	13.282	47.425

Gene name	Source
ADMP	Torlopp et al., 2014, <i>eLife</i>
ANGPTL4	ChEST714e17 (Source Bioscience)
ARL4C	ChEST901O6 (Source Bioscience)
ASTL	ChEST817d16 (Source Bioscience)
CDC42EP2	ChEST553I7 (Source Bioscience)
ChEST243a9	ChEST243a9 (Source Bioscience)
CHRD	Streit et al., 1998, Development
CHST1	ChEST540g14 (Source Bioscience)
CSTA	ChEST821f15 (Source Bioscience)
cVG1	Shah et al., 1997, Development
DKK1	Foley et al., 2000, Development
DLL1	This study
DOC2B	ChEST908m8 (Source Bioscience)
HEY1	Leimeister et al., 2000, Dev Biol
HHEX	Yatskievych et al., 1999, Mech Dev
HOXA2	ChEST671c8 (Source Bioscience)
IGFBP1	ChEST247h2 (Source Bioscience)
LFNG	Laufer et al., 1997, Nature
LHX1	ChEST389n6 (Source Bioscience)
LOC417741	ChEST714o24 (Source Bioscience)
LOC769139	ChEST675I8 (Source Bioscience)
LRIG1	ChEST252h22 (Source Bioscience)
MAFA	Torlopp et al., 2014, <i>eLife</i>
NKAIN4	ChEST110n2 (Source Bioscience)
NODAL	Levin et al., 1995, <i>Cell</i>
PITX2	Zhu et al., 1999, Curr Biol
RAB20	ChEST228j14 (Source Bioscience)
RNH1	ChEST73n20 (Source Bioscience)
SFRP1	Esteve et al., 2003, J Cell Sci
SPIK5	ChEST157b15 (Source Bioscience)
ST6GAL1	ChEST663h10 (Source Bioscience)
TBX6	Torlopp et al., 2014, <i>eLife</i>
VLDLR	ChEST263f6 (Source Bioscience)
WNT8C	Hume and Dodd, 1993, Development

Supplementary table 9. List of source of probes for *in situ* hybridisation