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斑马鱼胚胎中受 Nodal 信号调控基因的鉴定 *

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摘要 Nodal 信号在脊椎动物胚胎发育的中内胚层诱导、左右不对称性的建立、神经外胚层沿前后轴线的分化等方面起着重要的作用. 为鉴定受 Nodal 信号调控的基因，特别是那些转录因子基因，通过将来自 *squint* 过量表达、缺失 Nodal 信号的 *MZoep*突变体或野生型 30%外包期胚胎的 RNA 与 Affymetrix 斑马鱼寡核苷酸芯片杂交. 发现与野生型样本相比，在 *squint* 过量表达的样本中，265 个转录本的表达显著增强 ($\log_2$ ratio>1)，111 个转录本的表达显著减弱 ($\log_2$ ratio<−1)；在 *MZoep* 样本中，表达显著增强的 ($\log_2$ ratio>1)转录本有 1 495 个，表达显著减弱 ($\log_2$ ratio<−1) 的有 550 个. *squint* 过量表达使 26 个转录因子基因的表达增强，11 个转录因子基因的表达减弱；另一方面，*MZoep* 突变体中表达增强的转录因子基因为 69 个，表达减弱的转录因子基因为 30 个. 这些结果为进一步研究 Nodal 信号的转导机理和生物学功能提供了有益的数据.

关键词 斑马鱼，胚胎，Nodal，基因芯片，转录因子
学科分类号 Q7

Nodal 是 TGFβ 超家族的成员. 在脊椎动物胚胎发育过程中，Nodal 是关键的中胚层和内胚层诱导信号，在左右不对称发育中起着重要作用，也参与了神经外胚层沿前后轴线的分化[1]. 斑马鱼早期胚胎中表达有 2 个 Nodal 基因，即 *squint* 和 *cyclops*，它们在囊胚的中内胚层前体细胞中表达[2]. 给单细胞胚胎注射 *squint* 或 *cyclops* 的 mRNA，可以增强胚盾(embryonic shield，相当于爪蟾的组织中心)特异性基因和其他中内胚层基因的表达[2~5]. 在 *squint* 和 *cyclops* 基因都发生了突变的 *squint*；*cyclops* 双突变体胚胎中，原肠作用开始时缺少胚盾，发育到 24 h 时缺失几乎所有的中胚层和内胚层组织[6]，表明 Nodal 信号对于斑马鱼胚胎中内胚层的发育起决定性作用.

Nodal 信号的转导类似于其他的 TGFβ 家族成员[7]. 比较特殊的是，Nodal 信号分子与 Activin Ⅱ型和Ⅰ型受体的结合需要膜上的辅助受体——EGF-CFC 蛋白家族成员. 在斑马鱼上，Nodal 信号的辅助受体是 Oep，缺少母源和合子期表达的 Oep 的 *MZoep* 突变体胚胎，也不能发育出绝大部分的中胚层和内胚层组织[8,9].

Nodal 信号转导入核后，调控靶基因的表达，从而实现其生物学功能. 其靶基因中编码转录因子的基因，是 Nodal 信号生物学功能的下游执行者. 本项研究主要想通过基因芯片技术鉴定斑马鱼早期胚胎发育过程中受 Nodal 信号调控的转录因子基因，为将来深入研究 Nodal 信号实现其生物学功能的分子机制打下基础.

1 材料与方法

1.1 材料

野生型斑马鱼为 Tuebingen 品系. *oep* 突变品系为 *oep*^{tz257}. 用于 RNA 提取的 Trizol 为 Sigma 公司产品. 体外制备 mRNA 采用 Roche 公司的 Cap-Scribe 试剂盒. 斑马鱼基因芯片为 AFFYMETRIX 公司的产品.

1.2 方法

1.2.1 体外 mRNA 的合成. 先将含有靶基因的质粒 DNA 通过酶切直线化，按 Cap-Scribe 试剂盒的说明体外制备 mRNA，用无 RNA 酶活性的 DNA 酶处理除去 DNA 模板，然后用 Qiagen 公司的 RNeasy® Mini 试剂盒进行纯化，纯化后的 mRNA 溶于适当体积的去离子水中，用分光光度计测定

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浓度.

1.2.2 *MZoep* 突变体胚胎的获得.

第一步：*oep* 雌雄杂合体交配，所产胚胎在单细胞时注射 *oep* mRNA，待其成年后将雌雄分开.

第二步：将由第一步获得的雌鱼与 *oep* 雄鱼交配，所产生的胚胎如有 50%为 *MZoep* 突变体 (这类突变体可以从表型上分辨出来[9])，则说明此雌鱼是恢复正常的 *oep* 纯合突变体；将由第一步获得的雄鱼与 *oep* 雌鱼交配，所产生的胚胎表型如有 50%与 *MZoep* 突变体表型一样，则说明此雄鱼是恢复正常的 *oep* 纯合突变体.

第三步，将由第二步得到的恢复正常的雌雄 *oep* 纯合突变体交配，所产胚胎为 *MZoep* 突变体，收集 30%外包期的胚胎用于 RNA 的提取.

1.2.3 过量表达 *squint* 的胚胎. 用 *squint* mRNA 注射单细胞期野生型胚胎，注射剂量为每枚胚胎 5 pg. 当发育到 30%外包期时收集胚胎提取 RNA，同时保留部分胚胎在尾芽期观察以确定注射是否有效(*squint* mRNA 注射后的胚胎在尾芽期出现胚胎拉长的表型).

1.2.4 基因芯片杂交. 将从野生型胚胎、*squint* 过量表达的胚胎和 *MZoep* 突变体胚胎提取的 RNA 进行荧光标记，与 Affymetrix 公司的斑马鱼基因芯片杂交并检测信号. 该部分工作由上海晶泰生物技术有限公司完成. 每个样品杂交一张芯片.

2 结　果

在斑马鱼胚胎中，中胚层和内胚层在原肠期形成，但确定中、内胚层前体细胞的命运是发生在囊胚阶段[10]，Nodal 信号在此过程中起着关键的诱导作用[1]. 为了发现响应 Nodal 信号的早期靶基因，我们选择 3 种处在 30%外包期 (晚囊胚阶段[11]) 的胚胎样品，即过量表达 *squint* 的胚胎、缺失 Nodal 信号的 *MZoep* 突变体胚胎以及野生型胚胎. 将从这些胚胎提取的 RNA 与基因芯片杂交，即可发现受 Nodal 信号调控的早期表达基因.

本研究所利用的 Affymetrix 斑马鱼高密度基因芯片共包括约 14 900 个转录本（基因），芯片上每个转录本有 16 对 25 bp 长的寡核苷酸探针. 通过杂交发现，对于 *squint* mRNA 注射的样本，与野生型样本相比表达显著增强 ($\log_2$ ratio>1)的转录本有 265 个，表达显著减弱($\log_2$ ratio<–1)的转录本有 111 个，对于 *MZoep* 的样本，与野生样本相比表达显著增强 ($\log_2$ ratio>1)的转录本有 1 495 个，表达显著减弱($\log_2$ ratio<–1)的有 550 个，置信区间为 95% (图 1).

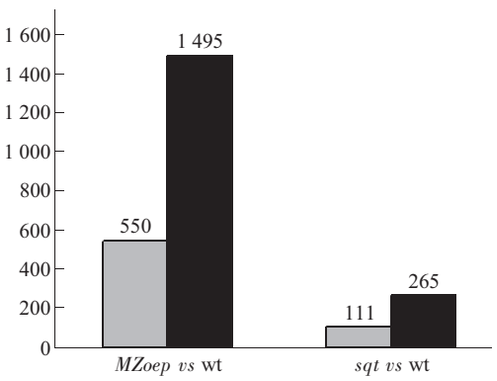

Fig. 1 The number of transcripts (genes) that were up- or down-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio < –1), with a 95% confidence interval

MZoep vs wt: The expression level change between *MZoep* and wt; *sqt vs* wt: The expression level change between *squint* mRNA-injected embryos and wt. □: Decrease; ■: Increase.

在上述差异表达的转录本中，包括了 *chordin*、*gsc*、*gata2*、*eve1* 等已知受 Nodal 信号调控基因的表达变化. 比如 *chordin* 和 *gsc* 在 *MZoep* 中表达极大减弱, 而在 *squint* 过量表达的胚胎中表达显著增强，过去的研究也已发现它们的表达受 Nodal 信号的正调控[2, 4, 6, 9, 12]. 基因芯片结果分析中还发现，*gata2*、*eve1* 和 *bmp2* 在 *squint* 过量表达的胚胎中表达量下降，而在 *MZoep* 胚胎中表达量增加，*fgf3*、*fgf8*、*bon*、*flh*、*ntl*、*dkk1*、*pitx2*、*tbx6* 和 *foxa2* 在 *squint* 过量表达的胚胎中表达量增加，而在 *MZoep* 胚胎中表达量下降，这些结果也与以前的报道一致[2, 4,9, 13~23]. 由此推测，我们的基因芯片结果是比较可信的.

根据 GO[24] (http://www.geneontology.org/) 对分子功能的定义 (即在分子水平描述基因产物的功能，一个基因产物可能有一个或多个功能)，本研究中的转录本共涉及 11 种分子功能，它们分别为：信号传导功能 (signal transducer activity)、转录调节功能 (transcription regulator activity)、催化功能 (catalytic activity)、抗氧化功能 (antioxidant activity)、结合功能 (binding)、翻译调节功能 (translation regulator activity)、转运功能 (transporter activity)、酶活性调节功能 (enzyme regulator activity)、结构分子功能 (structural molecular activity)、运动功能(motor activity)、化学吸引功能 (chemoattractant activity). 对于 *MZoep* 的样本，与野

生样本相比表达显著增强 ($\log_2$ ratio>1)的 1 495 个转录本中有注释的转录本为 565 个，根据上述分子功能分类，在有注释的转录本中，69 个有转录调节功能、54 个有信号传导功能、271 个有催化功能、5 个有抗氧化活性功能、379 个有结合功能、5 个有翻译调节功能、43 个有转运功能、22 个有酶活性调节功能、16 个有结构分子功能、4 个有运动活性功能. 另一方面，*MZoep* 胚胎与野生型胚胎相比表达显著减弱($\log_2$ ratio<−1)的 550 个转录本中，含有注释的转录本有 202 个，其中 30 个有转录调节功能、18 个有信号传导功能、87 个有催化功能、132 个有结合功能、6 个有翻译调节功能、7 个有转运功能、2 个有酶活性调节功能、10 个有结构分子功能、1 个有运动功能 (图 2).

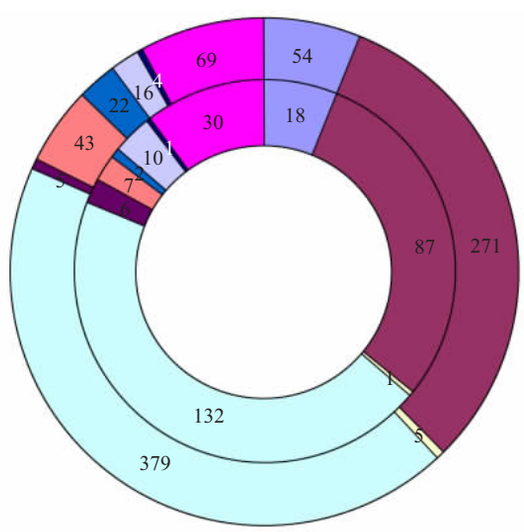

Fig. 2 Molecular function of annotated transcripts of *MZoep* embryos

The outer cycle indicates the transcripts that were up-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1); the inner cycles indicates the transcripts that were down-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1). ■: Signal transducer activity; ■: Antioxidant activity; ■: Translation regulator activity; ■: Enzyme regulator activity; ■: Motor activity; ■: Catalytic activity; ■: Binding; ■: Transporter activity; ■: Structural molecular activity; ■: Transcription regulator activity.

对于 *squint* mRNA 注射的样本，与野生型胚胎相比表达显著增强 ($\log_2$ ratio>1)的 265 个转录本中有注释为 118 个. 按分子功能分类，有注释的转录本包含了 9 类，分别为转录调节功能 26 个、信号传导功能 18 个、催化活性 44 个、结合功能 91 个、翻译调节功能 1 个、转运活性 2 个、酶调节功能 4 个、结构分子功能 1 个、运动活性的 1 个. 与野生型胚胎相比表达显著减弱($\log_2$ ratio<−1)的 111 个转录本中有注释的 49 个，这些有注释的基因按分子功能包含 9 类，即转录调节功能 11 个、信号传导功能 6 个、催化功能 14 个、结合功能 39 个、翻译调节功能 1 个、转运活性 4 个、结构分子功能 3 个、酶调节功能 1 个、化学吸引功能 1 个，如图 3 所示.

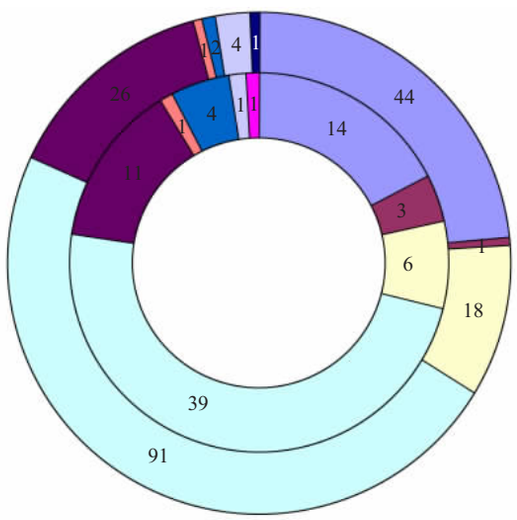

Fig. 3 Molecular function of annotated transcripts of *squint* mRNA-injected embryos

The outer cycle indicates the transcripts that were up-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1); the inner cycles indicates the transcripts that were down-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1). ■: Catalytic activity; ■: Signal transducer activity; ■: Transcription regulator activity; ■: Transporter activity; ■: Motor activity; ■: Structural molecular activity; ■: Binding; ■: Translation regulator activity; ■: Enzyme regulator activity; ■: Chemoattractant activity.

由于 Nodal 信号最终需要通过转录因子实现其对胚胎发育的调控，因此受 Nodal 信号调控的转录因子可能在介导和调节 Nodal 信号的转导及其生物学功能方面起重要作用. 为此，我们在表 1～4 中列

Table 1 List of transcription factor genes which were down-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1) in *squint*-overexpressing embryos

NO	Affymetrix probe identity	Annotated gene names
1	Dr.11698.1.S1_at	*tfap2c*
2	Dr.1691.12.S1_at	*sox3*
3	Dr.12624.1.S1_a_at	*irx1b*
4	Dr.12825.1.A1_at	*six7*
5	Dr.19888.1.S1_at	*ved*
6	Dr.4926.1.S1_at	*pbx4*
7	Dr.10428.1.S1_at	*irf7*
8	Dr.4845.1.A1_at	*mycn*
9	Dr.4845.2.S1_at	*mycy*
10	Dr.6769.1.A1_at	*creb3l3*
11	Dr.20010.8.A1_at	*sox3*

Table 2 List of transcription factor genes that were up-regulated significantly (log_2 ratio>1 or log_2 ratio<−1) in *squint*-overexpressing embryos

NO.	Affymetrix probe identity	Annotated gene names	NO.	Affymetrix probe identity	Annotated gene names
1	Dr.20027.1.S1_at	*nlz1*	14	Dr.289.1.S1_a_at	*gsc*
2	Dr.12603.1.S1_at	*irx7*	15	Dr.364.1.S1_at	*lhx1a*
3	Dr.20143.1.S1_at	*her3*	16	Dr.483.1.S1_at	*foxa2*
4	Dr.25055.1.A1_s_at	*lhx1a*	17	Dr.546.1.S1_at	*vsx2*
5	Dr.25405.1.A1_at	*sox19b*	18	Dr.5771.1.S1_at	*otx1*
6	Dr.7103.1.S1_at	*Id3*	19	Dr.587.1.S1_at	*foxb1.2*
7	Dr.1307.1.S1_at	*foxa3*	20	Dr.8282.2.S1_a_at	*irx3a.*
8	Dr.1468.1.S1_at	*ntl*	21	DrAffx.1.67.S1_at	*emx1*
9	Dr.15719.1.S1_at	*foxc1a*	22	Dr.13964.3.A1_at	Si:ch211-260g14.3
10	Dr.1680.1.S1_at	*meis3*	23	Dr.16048.1.S1_at	zgc:55680
11	Dr.18302.1.S1_at	*og9x*	24	Dr.7710.1.A1_at	zgc:92106
12	Dr.224.1.S1_at	*tbx6*	25	Dr.24992.1.A1_at	zgc:92774
13	Dr.277.1.S1_at	*lhx1b*	26	Dr.9243.1.A1_at	zgc:92434

Table 3 List of transcription factor genes that were down-regulated significantly (log_2 ratio>1 or log_2 ratio<−1) in *MZoep* mutant embryos

NO.	Affymetrix probe identity	Annotated gene names	NO.	Affymetrix probe identity	Annotated gene names
1	Dr.12386.1.S1_x_at	LOC565864; *msgn1*	16	Dr.483.1.S1_at	*foxa2*
2	Dr.1462.1.S1_at	*her1*	17	Dr.590.1.S1_at	*foxd3*
3	Dr.25055.1.A1_s_at	*lhx1a*	18	Dr.8084.1.S1_at	*bon*
4	Dr.19467.1.A1_at	*hes6*	19	Dr.8202.2.S1_a_at	*pitx2a*
5	Dr.352.1.S1_at	*flh*	20	Dr.8301.1.S1_a_at	*hsf1*
6	Dr.3696.1.S1_at	*her7*	21	Dr.8301.4.S1_a_at	*hsf1*
7	Dr.8162.1.S1_at	*mespa*	22	Dr.8282.2.S1_a_at	*irx3a*
8	Dr.8197.1.S1_at	*atoh2b*	23	Dr.10326.1.S1_at	*junb*
9	Dr.1307.1.S1_at	*foxa3*	24	Dr.14771.2.S1_at	*foxo3a*
10	Dr.1468.1.S1_at	*ntl*	25	.Dr.2401.1.A1_at	*usf2*
11	Dr.15719.1.S1_at	*foxc1a*	26	Dr.1630.1.S1_at	*gtf2h2*
12	Dr.18302.1.S1_at	*og9x*	27	Dr.7710.1.A1_at	zgc:92106
13	Dr.224.1.S1_at	*tbx6,*	28	Dr.12595.1.S1_at	*hsf2*
14	Dr.289.1.S1_a_at	*gsc*	29	Dr.20342.1.S1_at	zgc:55543
15	Dr.364.1.S1_at	*lhx1a*	30	Dr.5284.1.S1_at	*taf7*

Table 4 List of transcription factor genes that were up-regulated significantly (log_2 ratio>1 or log_2 ratio<−1) in *MZoep* mutant embryos

NO.	Affymetrix probe identity	Annotated gene names	NO.	Affymetrix probe identity	Annotated gene names
1	Dr.10723.1.S1_at	*tbr1*	16	Dr.15663.1.S1_at	*cebpg*
2	Dr.11727.1.S1_at	*esr2a*	17	Dr.14.1.S1_at	*pouc*
3	Dr.1216.1.A1_at	*atf7b*	18	Dr.145.1.S1_at	*dlx6a*
4	Dr.12603.1.S1_at	*irx7*	19	Dr.15714.1.S1_at	*hoxd9a*
5	Dr.13338.1.S1_at	*gbx1*	20	Dr.16024.1.A1_at	*tbpl2*
6	Dr.1691.10.S1_at	*her8a*	21	Dr.16111.1.S1_at	*sdccag33*
7	Dr.18462.1.A1_at	*irx4b*	22	Dr.179.1.S2_at	*tbx16*
8	Dr.25210.1.S1_at	*arntl1b*	23	Dr.19893.1.S1_at	*znf503*
9	Dr.4597.1.S1_at	*mxi1*	24	Dr.20969.1.S1_at	*foxi1*
10	Dr.4845.2.S1_at	*mycn*	25	Dr.21063.1.A1_at	*bapx1*
11	Dr.546.1.S1_at	*vsx2*	26	Dr.23455.1.S1_at	*pax7*
12	Dr.57.1.S1_at	*pou50*	27	Dr.24779.1.S1_at	*msxb*
13	Dr.7608.2.S1_at	*jun*	28	Dr.25775.1.S1_at	*irx4a*
14	Dr.7815.1.S1_at	*snap25a*	29	Dr.284.2.A1_a_at	*otx1*
15	Dr.12836.2.A1_at	*id2b*	30	Dr.356.1.S1_at	*gata2*

Continued

NO.	Affymetrix probe identity	Annotated gene names	NO.	Affymetrix probe identity	Annotated gene names
31	Dr.454.1.S1_at	*thra*	51	Dr.7758.1.A1_at	zgc:76986
32	Dr.463.1.S1_at	*her5*	52	Dr.15279.1.S1_s_at	*trpc4apb*
33	Dr.558.1.S1_at	*vsx1*	53	Dr.13012.1.S1_at	*nfkb2*
34	Dr.12986.1.A1_at	*fos*	54	Dr.15437.1.S1_at	zgc:56325
35	Dr.5736.1.S1_at	*hoxd12a*	55	Dr.16631.1.A1_at	zgc:101119
36	Dr.616.1.S1_at	*six3a*	56	Dr.16669.1.S1_at	zgc:65854
37	Dr.8070.1.S1_at	*dbx1a*	57	Dr.16743.1.A1_at	zgc:65895
38	Dr.8215.1.A1_at	*sox21a*	58	Dr.17623.1.S1_at	zgc:63854
39	Dr.8231.1.S1_at	*tbx20*	59	Dr.20994.1.S1_s_at	*meis4.1a*
40	Dr.8232.1.S1_at	*hey2*	60	Dr.2313.1.S1_at	zgc:56067
41	Dr.1031.1.S1_at	zgc:110158	61	Dr.24992.1.A1_at	zgc:92774
42	Dr.12836.1.S1_at	*id2b*	62	Dr.4845.2.S1_at	zgc:85706
43	Dr.13964.1.S1_at	si:ch211-260g14.3	63	Dr.7608.2.S1_at	zgc:65863
44	Dr.15418.1.S1_at	wu:fc23f06	64	Dr.9243.1.A1_at	zgc:92434
45	Dr.15539.1.A1_at	zgc:111879	65	Dr.599.1.S1_at	*ldb2*
46	Dr.16499.1.A1_at	zgc:113038	66	Dr.8118.1.A1_at	*otp*
47	Dr.19969.1.S1_at	LOC407678	67	Dr.8167.2.S1_at	*srf*
48	Dr.20140.1.A1_at	zgc:111879	68	Dr.8325.1.S1_at	*mxtx2*
49	Dr.5645.1.S1_at	si:dkey-12h9.10	69	Dr.9994.1.A1_at	*lhx8*
50	Dr.7345.1.S1_at	zgc:113424, LOC554876			

出了所有受 Nodal 信号正调控或负调控的具有潜在转录调节功能的基因，希望能为感兴趣的研究者提供有益的信息.

3 讨　论

限于成本的考虑，本项基因芯片实验未设置重复. 但是，我们注意到，本次实验中所发现的许多受 Nodal 信号调控的基因在以往的研究中已有报道，说明我们得到的结果还是有较高的可信性. 对于那些与 Nodal 信号的关系还未见报道的基因，尚需进一步的实验加以验证.

从本次实验结果来看，注射了 *squint* mRNA 的样本与野生型样本表达显著增强 ($\log_2$ ratio>1)的转录本有 265 个，表达显著减弱($\log_2$ ratio<−1)的转录本有 111 个，而对于 *MZoep* 突变体样本，与野生样本相比表达显著增强 ($\log_2$ ratio>1)的转录本有 1 495 个，表达显著减弱($\log_2$ ratio<−1)的有 550 个，增强与减弱的基因数均比注射 *squint* mRNA 的样本多很多(图 1). 由此可以看出，当 Nodal 信号完全缺失时，可以造成更多的基因异常表达. 考虑到 *MZoep* 突变体缺失绝大部分中胚层和内胚层组织，因此受影响的基因更多是可以理解的.

从实验结果还发现一个现象，在 *MZoep* 胚胎中表达显著增强 ($\log_2$ ratio>1)的转录本与 *squint* mRNA 注射的样本中表达显著减弱($\log_2$ ratio<−1)的转录本有 11 个是一样的 (表 5)，而 *MZoep* 胚胎中表达降低大于 2 倍的转录本与 *squint* mRNA 注射的样本中表达显著增强 ($\log_2$ ratio>1)的转录本中的有 27 个是一样的 (表 6)，这一结果很好解释. 不过奇怪的是 *MZoep* 样本中表达显著增强 ($\log_2$ ratio >1)的转录本与 *squint* mRNA 注射的样本中表达显著增强 ($\log_2$ ratio>1)的转录本中有 73 个是一样，见表 7，*MZoep* 样本中表达显著减弱($\log_2$ ratio<−1)的转录本与 *squint* mRNA 注射的样本中表达显著减弱

Table 5 The intersection of transcripts that were up-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1) in *MZoep* mutant embryos and were down-regulated significantly ($\log_2$ ratio>1 or $\log_2$ ratio<−1) in *squint*-overexpressing embryos

NO.	Affymetrix probe identity	Gene symbol or gene title
1	Dr.16873.1.A1_at	
2	Dr.24000.1.A1_at	
3	Dr.25497.1.S1_at	*szl*
4	Dr.26461.1.S1_at	zgc:63947
5	Dr.4845.1.A1_at	*mycn*
6	Dr.4845.2.S1_at	*mycn*
7	Dr.4932.1.S1_at	*anxa4*
8	Dr.568.1.S1_at	*bmp2b*
9	Dr.7919.1.S1_at	*bambi*
10	Dr.822.1.S3_at	*cxcl12a*
11	Dr.9976.1.S1_at	*klf2b*

Table 6 The intersection of transcripts that were up-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *squint*-overexpressing embryos and were down-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *MZoep* mutant embryos

NO.	Affymetrix probe identity	Gene symbol or gene title	NO.	Affymetrix probe identity	Gene symbol or gene title
1	Dr.1137.1.A1_at	CDNA clone IMAGE:7403183	15	Dr.289.1.S1_a_at	*gsc*
2	Dr.11380.1.A1_at		16	Dr.364.1.S1_at	*lhx1a*
3	Dr.1307.1.S1_at	*foxa3*	17	Dr.3827.1.A1_at	LOC557315
4	Dr.13384.1.S1_at	*chd*	18	Dr.389.1.S1_at	*wnt5b*
5	Dr.1468.1.S1_at	*ntl*	19	Dr.483.1.S1_at	*foxa2*
6	Dr.15719.1.S1_at	*foxc1a*	20	Dr.609.1.S1_at	*pcdh8*
7	Dr.17776.1.A1_at	*tph1l*	21	Dr.6501.1.S1_at	sb:cb825
8	Dr.18302.1.S1_at	*og9x*	22	Dr.7710.1.A1_at	zgc:92106
9	Dr.18614.1.A1_at	transcribed locus	23	Dr.8056.1.S1_at	*dkk1*
10	Dr.19144.1.A1_at	*p4ha2*	24	Dr.8208.1.S1_at	*lefty1*
11	Dr.224.1.S1_at	*tbx6*	25	Dr.8282.2.S1_a_at	*irx3a*
12	Dr.23570.1.A1_at	wu:fb13b10	26	Dr.9304.1.S1_at	zgc:55423 LOC554932 LOC558854
13	Dr.24443.1.A1_at	*lhx1a*	27	DrAffx.1.2.S1_at	*bhik*
14	Dr.25055.1.A1_s_at	*lhx1a*			

Table 7 The intersection of transcripts that were down-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *MZoep* mutant embryos and were down-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *squint*-overexpressing embryos

NO.	Affymetrix probe identity	Gene symbol or gene title
1	Dr.11130.1.A1_at	zgc:101559
2	Dr.11708.1.S1_at	zgc:55580
3	Dr.12425.1.S1_at	zgc:92533
4	Dr.12425.1.S1_x_at	zgc:92533
5	Dr.13267.1.A1_at	Transcribed locus
6	Dr.14115.1.S1_at	Transcribed locus
7	Dr.15438.1.A1_at	zgc:63657
8	Dr.18080.1.S1_at	*dedaf*
9	Dr.19412.1.A1_at	Transcribed locus
10	Dr.20648.1.A1_at	Transcribed locus
11	Dr.21961.1.A1_at	wu:fc87g11
12	Dr.22471.1.A1_at	wu:fe38h02
13	Dr.24708.1.A1_at	transcribed locus
14	Dr.25140.2.S1_at	*icn*
15	Dr.25140.2.S1_a_at	*icn*
16	Dr.25140.2.S1_x_at	*icn*
17	Dr.25291.1.S1_at	D93 mRNA, 3'UTR, partial sequence
18	Dr.25322.1.S1_at	*lin7c*
19	Dr.26107.1.A1_at	
20	Dr.26197.1.S1_at	Transcribed locus, weakly similar to XP_001059414.1 PREDICTED
21	Dr.26197.2.A1_at	Transcribed locus, weakly similar to XP_001059414.1 PREDICTED
22	Dr.263.2.S1_at	*plasticin*
23	Dr.2646.1.A1_at	wu:fb13b04
24	Dr.4128.1.A1_at	LOC557079
25	Dr.4198.1.S1_at	*mhc1ufa*
26	Dr.4260.1.A1_at	Transcribed locus, weakly similar to XP_319290.2 ENSANGP00000012286
27	Dr.5322.1.S1_at	LOC555790
28	Dr.6286.1.A1_at	Transcribed locus
29	Dr.6913.1.S1_at	*c12orf2*
30	Dr.7731.1.A1_at	
31	Dr.8110.1.S1_at	*bmp7*

Table 8 The intersection of transcripts that were up-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *MZoep* mutant embryos and were up-regulated significantly (log_2 ratio>1 or log_2 ratio<–1) in *squint*-overexpressing embryos

NO.	Affymetrix probe identity	Gene symbol or gene title
1	Dr.10341.1.S1_at	*fzd9*
2	Dr.10562.1.A1_at	*olfm2*
3	Dr.10623.1.A1_at	*fzd9*
4	Dr.11176.1.A1_s_at	*aanat1*
5	Dr.11469.1.A1_a_at	Hypothetical protein LOC554817
6	Dr.11847.1.S1_s_at	*notch1a*
7	Dr.12259.1.S1_at	*btg4*
8	Dr.12341.1.A1_at	Transcribed locus
9	Dr.12399.1.S1_at	*mst1*
10	Dr.12603.1.S1_at	*irx7*
11	Dr.12731.2.A1_x_at	Transcribed locus, weakly similar to NP_663446.1
12	Dr.13137.1.A1_at	Transcribed locus
13	Dr.13157.1.A1_at	Transcribed locus
14	Dr.13585.1.A1_at	
15	Dr.13964.3.A1_at	si:ch211-260g14.3
16	Dr.13985.1.A1_at	
17	Dr.14244.1.S1_at	zgc:55863
18	Dr.14488.1.A1_at	Transcribed locus
19	Dr.14713.1.A1_at	Transcribed locus
20	Dr.14821.1.A1_at	*kctd12.1*
21	Dr.15045.1.S1_at	zgc:112262
22	Dr.1519.1.S1_at	*rab6a* LOC554520
23	Dr.15592.1.A1_at	LOC555637
24	Dr.15729.1.S1_at	DnaJ (Hsp40) homolog, subfamily A, member 3A hypothetical protein LOC55486725
25	Dr.15984.1.S1_at	zgc:112426
26	Dr.16696.1.S1_at	LOC556629
27	Dr.16698.1.A1_at	LOC564598
28	Dr.168.1.A1_at	wu:fj12e10
29	Dr.17145.1.S1_at	
30	Dr.17225.1.S1_at	
31	Dr.17242.1.A1_at	zgc:76924
32	Dr.17260.1.A1_at	LOC572584
33	Dr.17305.1.A1_at	Similar to F-box protein 15 LOC556635 LOC566970
34	Dr.17450.1.A1_at	zgc:63663
35	Dr.17665.3.S1_a_at	Hypothetical protein FLJ11011-like (H. sapiens)
36	Dr.1991.1.A1_at	zgc:66417
37	Dr.20108.1.S1_a_at	*qars*
38	Dr.20924.1.S1_at	zgc:55889
39	Dr.21562.1.A1_at	*dlg5*
40	Dr.22187.1.A1_at	Transcribed locus, weakly similar to XP_418469.1 PREDICTED: similar to zinc finger protein 27
41	Dr.22945.1.S1_at	zgc:56445
42	Dr.24062.1.A1_at	wu:fj42c06
43	Dr.24992.1.A1_at	zgc:92774
44	Dr.25341.1.A1_at	zgc:63631
45	Dr.25498.1.S1_at	*bzw1*
46	Dr.25673.1.S1_at	*cdc25*
47	Dr.25748.1.A1_at	
48	Dr.26111.1.A1_at	Transcribed locus
49	Dr.26317.1.A1_at	Transcribed locus
50	Dr.2919.1.S1_at	wu:fb40b03
51	Dr.3373.1.S1_at	*col18a1*

Continued

NO.	Affymetrix probe identity	Gene symbol or gene title
52	Dr.3730.1.A1_at	zgc:86909
53	Dr.3746.1.A1_a_at	zgc:110840
54	Dr.3785.1.S1_at	
55	Dr.4367.1.A1_at	wu:fc38g09
56	Dr.546.1.S1_at	*vsx2*
57	Dr.5477.1.S1_at	*ptma*
58	Dr.5802.1.S1_at	Similar to LR8 protein LOC563410
59	Dr.6007.1.S1_at	*cdh1*
60	Dr.610.2.S1_at	Similar to Heat shock protein HSP 90-alpha (HSP 8) DKEY-241L7.8
61	Dr.6424.1.A1_at	
62	Dr.6680.1.S1_at	zgc:55587
63	Dr.6789.1.A1_at	wu:fj59h01
64	Dr.6948.1.A1_at	
65	Dr.7822.1.A1_at	*atp6ap2*
66	Dr.79.1.A1_at	sb:cb382
67	Dr.8587.1.A2_at	*igfbp1*
68	Dr.9237.1.A1_at	wu:fc19a02
69	Dr.9240.1.A1_at	Transcribed locus, weakly similar to XP_001058034.1 PREDICTED
70	Dr.9243.1.A1_at	zgc:92434
71	DrAffx.1.13.S1_at	*ankrd6*
72	DrAffx.1.20.S1_at	zgc:113947
73	DrAffx.1.33.S1_at	zgc:91787

($\log_2$ ratio<–1)的转录本中有 31 个是一样的，见表 8，出现这种现象可能的解释是：有些受 Nodal 信号调控的基因是反馈自调控的，一般情况下，这些基因的表达处于一种动态平衡的状态下，在一定的范围内，如果 Nodal 信号增强或减弱，这些基因表达跟着增强或减弱，但是，当 Nodal 信号的增强或减弱超过了一定的范围即超过一定的阈值时，机体为了维持正常的生长需要，使这些基因仍然处于动态平衡状态之下，这些基因会相反地减弱或增强，这是机体的一种自我保护的功能，*MZoep* 突变体是 Nodal 信号完全缺失的样本，属于一种极端的生长异常情况，在这种情况下，某些基因会出现与 Nodal 信号部分增强时表达一致的情况，至于这种解释是否正确还有待于进一步的实验对这些基因的验证. 当然这种现象的出现还可能是因某种不被我们了解的机制存在.

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Identification of Nodal-regulated Genes in Zebrafish Embryos*

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Abstract Nodal signals play important roles in mesendoderm induction, establishment of left-right asymmetry and anteroposterior patterning of the neuroectoderm during vertebrate embryogenesis. It is aimed at identifying Nodal-regulated genes in zebrafish embryos, particularly those encoding transcription factors. A (Affymetrix) genechip analysis was performed using RNAs derived from embryos injected with *squint* mRNA, *MZoep* mutant embryos that are deficient in Nodal signaling, and wildtype embryos at the 30% epiboly stage. Transcripts (genes) with at least two-fold changes in expression level between wildtype and the other samples were identified. In *squint* mRNA-injected embryos, 265 transcripts show an increased expression level and 111 have a decreased expression level; in *MZoep* embryos, the expression of 1 495 transcripts increases while 550 transcripts express at a decreased level. Furthermore, overexpression of *squint* causes increased expression of 26 and decreased expression of 11 annotated transcription factor genes; in *MZoep* embryos, the number of transcription factor genes showing an increase and decrease of expression are 69 and 30, respectively. These results would provide useful information for further studying mechanisms and biological functions of Nodal signal transduction.

Key words zebrafish, embryos, Nodal, genechip, transcription factor

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