Developmental Biology


European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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21/06/2020
Introduction

Reactome is an open-source, open-access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 73

This document contains 11 pathways (see Table of Contents)
As early steps towards capturing the array of processes by which a fertilized egg gives rise to the diverse tissues of the body, examples of ten processes have been annotated. Aspects of two processes involved in most developmental processes, transcriptional regulation of pluripotent stem cells, and activation of HOX genes during differentiation are annotated. More specialized processes include nervous system development, aspects of the roles of cell adhesion molecules in axonal guidance and myogenesis, of transcriptional regulation in pancreatic beta cell, transcriptional regulation of granulopoiesis, and transcriptional regulation of white adipocyte differentiation, molecular events of "nodal" signaling, LGI-ADAM interactions, and keratinization.

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Neurogenesis is the process by which neural stem cells give rise to neurons, and occurs both during embryonic and perinatal development as well as in specific brain lineages during adult life (reviewed in Götz and Huttner, 2005; Yao et al, 2016; Kriegstein and Alvarez-Buylla, 2009).

**Literature references**


**Editions**

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Myogenesis, the formation of muscle tissue, is a complex process involving steps of cell proliferation mediated by growth factor signaling, cell differentiation, reorganization of cells to form myotubes, and cell fusion. Here, one regulatory feature of this process has been annotated, the signaling cascade initiated by CDO (cell-adhesion-molecule-related/downregulated by oncogenes) and associated co-receptors.

CDO/Cdon is a type I transmembrane multifunctional co-receptor consisting of five immunoglobulin and three fibronectin type III (FNIII) repeats in the extracellular domain, and an intracellular domain with no identifiable motifs. It has been implicated in enhancing muscle differentiation in promyogenic cells. CDO exert its promyogenic effects as a component of multiprotein complexes that include the closely related factor Boc, the Ig superfamily receptor neogenin and its ligand netrin-3, and the adhesion molecules N- and M-cadherin. CDO modulates the Cdc42 and p38 mitogen-activated protein kinase (MAPK) pathways via a direct association with two scaffold-type proteins, JLP and Bnip-2, to regulate activities of myogenic bHLH factors and myogenic differentiation. CDO activates myogenic bHLH factors via enhanced heterodimer formation, most likely by inducing hyper-phosphorylation of E proteins.

Myogenic basic helix-loop-helix (bHLH) proteins are master regulatory proteins that activate the transcription of many muscle-specific genes during myogenesis. These myogenic bHLH proteins also referred to as MyoD family includes four members, MyoD, myogenin, myf5 and MRF4. These myogenic factors dimerize with E-proteins such as E12/E47, ITF-2 and HEB to form heterodimeric complexes that bind to a conserved DNA sequence known as the E box, which is present in the promoters and enhancers of most muscle-specific genes. Myocyte enhancer binding factor 2 (MEF2), which is a member of the MADS box family, also plays an important role in muscle differentiation. MEF2 activates transcription by binding to the consensus sequence, called the MEF2-binding site, which is also found in the control regions of numerous muscle-specific genes. MEF2 and myogenic bHLH proteins synergistically activate expression of muscle-specific genes via protein-protein interactions between DNA-binding domains of these heterologous classes of transcription factors. Members of the MyoD and MEF2 family of transcription factors associate combinatorially to control myoblast specification, differentiation and proliferation.
Literature references


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The normal development of the pancreas during gestation has been intensively investigated over the past decade especially in the mouse (Servitja and Ferrer 2004; Chakrabarti and Mirmira 2003). Studies of genetic defects associated with maturity onset diabetes of the young (MODY) has provided direct insight into these processes as they take place in humans (Fajans et al. 2001). During embryogenesis, committed epithelial cells from the early pancreatic buds differentiate into mature endocrine and exocrine cells. It is helpful to schematize this process into four consecutive cellular stages, to begin to describe the complex interplay of signal transduction pathways and transcriptional networks. The annotations here are by no means complete - factors in addition to the ones described here must be active, and even for the ones that are described, only key examples of their regulatory effects and interactions have been annotated.

It is also important to realize that in the human, unlike the mouse, cells of the different stages can be present simultaneously in the developing pancreas and the linear representation of these developmental events shown here is an over-simplification of the actual developmental process (e.g., Sarkar et al. 2008).

The first stage of this process involves the predifferentiated epithelial cells of the two pancreatic anlagen that arise from the definitive endoderm at approximately somite stages 11-15 and undergo budding from somite stages 20-22. This period corresponds to gestational days 8.75-9.5 in the mouse, and 26 in the human.

Pancreatic buds subsequently coalesce to form a single primitive gland, while concomitantly a ductal tree lined by highly proliferative epithelial cells is formed. A subset of such epithelial cells is thought to differentiate into either endocrine or acinar exocrine cells. A third cellular stage is defined by the endocrine-committed progenitors that selectively express the basic helix-loop-helix transcription factor NEUROG3. NEUROG3 is known to activate a complex transcriptional network that is essential for the spe-
cification of endocrine cells. Many transcription factors that are activated by NEUROG3 are also involved in islet-subtype cellular specification and in subsequent stages of differentiation of endocrine cells. This transient cellular stage thus leads to the generation of all known pancreatic endocrine cells, including insulin-producing beta-cells, and glucagon-producing alpha cells, the final stage of this schematic developmental process.

The diagram below summarizes interactions that take place between transcription factors and transcription factor target genes during these cellular stages, and shows cases where there is both functional evidence that a transcription factor is required for the target gene to be expressed, and biochemical evidence that this interaction is direct. We also describe instances where a signaling pathway is known to regulate a transcription factor gene in this process, even if the intervening signaling pathway is not fully understood.

**Literature references**


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Signaling by NODAL

Location: Developmental Biology

Stable identifier: R-HSA-1181150

Compartments: cytosol, extracellular region, nucleoplasm, plasma membrane

Signaling by NODAL is essential for patterning of the axes of the embryo and formation of mesoderm and endoderm (reviewed in Schier 2009, Shen 2007). The NODAL proprotein is secreted and cleaved extracellularly to yield mature NODAL. Mature NODAL homodimerizes and can also form heterodimers with LEFTY1, LEFTY2, or CERBERUS, which negatively regulate NODAL signaling. NODAL also forms heterodimers with GDF1, which increases NODAL activity. NODAL dimers bind the NODAL receptor comprising a type I Activin receptor (ACVR1B or ACVR1C), a type II Activin receptor (ACVR2A or ACVR2B), and an EGF-CFC coreceptor (CRIPTO or CRYPTIC). After binding NODAL, the type II activin receptor phosphorylates the type I activin receptor which then phosphorylates SMAD2 and SMAD3 (R-SMADs). Phosphorylated SMAD2 and SMAD3 form hetero-oligomeric complexes with SMAD4 (CO-SMAD) and transit from the cytosol to the nucleus. Within the nucleus the SMAD complexes interact with transcription factors such as FOXH1 to activate transcription of target genes.

Literature references


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Adipogenesis is the process of cell differentiation by which preadipocytes become adipocytes. During this process the preadipocytes cease to proliferate, begin to accumulate lipid droplets and develop morphologic and biochemical characteristics of mature adipocytes such as hormone responsive lipogenic and lipolytic programs. The most intensively studied model system for adipogenesis is differentiation of the mouse 3T3-L1 preadipocyte cell line by an induction cocktail containing mitogens (insulin/IGF1), glucocorticoid (dexamethasone), an inducer of cAMP (IBMX), and fetal serum (Cao et al. 1991, reviewed in Farmer 2006). More recently additional cellular models have become available to study adipogenesis that involve almost all stages of development (reviewed in Rosen and MacDougald 2006). In vivo knockout mice lacking putative adipogenic factors have also been extensively studied. Human pathways are traditionally inferred from those discovered in mouse but are now beginning to be validated in cellular models derived from human adipose progenitors (Fischer-Posovszky et al. 2008, Wdziekonski et al. 2011).

Adipogenesis is controlled by a cascade of transcription factors (Yeh et al. 1995, reviewed in Farmer 2006, Gesta et al. 2007). One of the first observable events during adipocyte differentiation is a transient increase in expression of the CEBPB (CCAAT/Enhancer Binding Protein Beta, C/EBPB) and CEBPD (C/EBPD) transcription factors (Cao et al. 1991, reviewed in Lane et al. 1999). This occurs prior to the accumulation of lipid droplets. However, it is the subsequent inductions of CEBPA and PPARG that are critical for morphological, biochemical and functional adipocytes.

Ectopic expression of CEBPB alone is capable of inducing substantial adipocyte differentiation in fibroblasts while CEBPD has a minimal effect. CEBPB is upregulated in response to intracellular cAMP (possibly via pCREB) and serum mitogens (possibly via Krox20). CEBPD is upregulated in response to gluco-
corticoids. The exact mechanisms that upregulate the CEBPs are not fully known.

CEBPB and CEBPD act directly on the Peroxisome Proliferator-activated Receptor Gamma (PPARG) gene by binding its promoter and activating transcription. CEBPB and CEBPD also directly activate the EBF1 gene (and possibly other EBFs) and KLF5 (Jimenez et al. 2007, Oishi 2005). The EBF1 and KLF5 proteins, in turn, bind, and activate the PPARG promoter. Other hormones, such as insulin, affect PPARG expression and other transcription factors, such as ADD1/SREBP1c, bind the PPARG promoter. This is an area of ongoing research.

During adipogenesis the PPARG gene is transcribed to yield 2 variants. The adipogenic variant 2 mRNA encodes 30 additional amino acids at the N-terminus compared to the widely expressed variant 1 mRNA.

PPARG encodes a type II nuclear hormone receptor (remains in the nucleus in the absence of ligand) that forms a heterodimer with the Retinoid X Receptor Alpha (RXRA). The heterodimer was initially identified as a complex regulating the aP2/FABP4 gene and named ARF6 (Tontonoz et al. 1994).

The PPARG:RXRA heterodimer binds a recognition sequence that consists of two hexanucleotide motifs (DR1 motifs) separated by 1 nucleotide. Binding occurs even in the absence of ligands, such as fatty acids, that activate PPARG. In the absence of activating ligands, the PPARG:RXRA complex recruits corepressors of transcription such as SMRT/NCoR2, NCoR1, and HDAC3 (Tontonoz and Spiegelman 2008).

Each molecule of PPARG can bind 2 molecules of activating ligands. Although, the identity of the endogenous ligands of PPARG is unknown, exogenous activators include fatty acids and the thiazolidinedione class of antidiabetic drugs (reviewed in Berger et al. 2005, Heikkinen et al. 2007, Lemberger et al. 1996). The most potent activators of PPARG in vitro are oxidized derivatives of unsaturated fatty acids. Upon binding activating ligands PPARG causes a rearrangement of adjacent factors: Corepressors such as SMRT/NCoR2 are lost and coactivators such as TIF2, PRIP, CBP, and p300 are recruited (Tontonoz and Spiegelman). PPARG also binds directly to the TRAP220 subunit of the TRAP/Mediator complex that recruits RNA polymerase II. Thus binding of activating ligand by PPARG causes transcription of PPARG target genes.

Targets of PPARG include genes involved in differentiation (PGAR/HFARP, Perilipin, aP2/FABP4, CEBPA), fatty acid transport (LPL, FAT/CD36), carbohydrate metabolism (PEPCK-C, AQP7, GK, GLUT4 (SLC2A4)), and energy homeostasis (LEPTIN and ADIPONECTIN) (Perera et al. 2006).

Within 10 days of differentiation CEBPB and CEBPD are no longer located at the PPARG promoter. Instead CEBPA is present. EBF1 and PPARG bind the CEBPA promoter and activate transcription of CEBPA, one of the key transcription factors in adipogenesis. A current hypothesis posits a self-reinforcing loop that maintains PPARG expression and the differentiated state: PPARG activates CEBPA and CEBPA activates PPARG. Additionally EBF1 (and possibly other EBFs) activates CEBPA, CEBPA activates EBF1, and EBF1 activates PPARG.

**Literature references**


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Transcriptional regulation of pluripotent stem cells

Location: Developmental Biology

Stable identifier: R-HSA-452723

Compartments: cytosol, nucleoplasm


Pluripotency is maintained by a self-reinforcing loop of transcription factors (Boyer et al. 2005, Rao et al. 2006, Matoba et al. 2006, Player et al. 2006, Babaie et al. 2007, Sun et al. 2008, Assou et al. 2009, reviewed in Kashyap et al. 2009, reviewed in Dejosez and Zwaka 2012). In vivo, initiation of pluripotency may depend on maternal factors transmitted through the oocyte (Assou et al. 2009) and on DNA demethylation in the zygote (recently reviewed in Seisenberger et al. 2013) and hypoxia experienced by the blastocyst in the reproductive tract before implantation (Forristal et al. 2010, reviewed in Mohyeldin et al. 2010). In vitro, induced pluripotency may initiate with demethylation and activation of the promoters of POU5F1 (OCT4) and NANOG (Bhutani et al. 2010). Hypoxia also significantly enhances conversion to pluripotent stem cells (Yoshida et al. 2009). POU5F1 and NANOG, together with SOX2, encode central factors in pluripotency and activate their own transcription (Boyer et al 2005, Babaie et al. 2007, Yu et al. 2007, Takahashi et al. 2007). The autoactivation loop maintains expression of POU5F1, NANOG, and SOX2 at high levels in stem cells and, in turn, complexes containing various combinations of these factors (Remenyi et al. 2003, Lam et al. 2012) activate the expression of a group of genes whose products are associated with

Comparisons between human and mouse embryonic stem cells must be made with caution and for this reason inferences from mouse have been used sparingly in this module. Human ESCs more closely resemble mouse epiblast stem cells in having inactivated X chromosomes, flattened morphology, and intolerance to passaging as single cells (Hanna et al. 2010). Molecularly, human ESCs differ from mouse ESCs in being maintained by FGF and Activin/Nodal/TGFbeta signaling rather than by LIF and canonical Wnt signaling (Greber et al. 2010, reviewed in Katoh 2011). In human ESCs POU5F1 binds and directly activates the FGF2 gene, however Pou5f1 does not activate Fgf2 in mouse ESCs (reviewed in De Los Angeles et al. 2012). Differences in expression patterns of KLF2, KLF4, KLF5, ESRRB, FOXD3, SOCS3, LIN28, NODAL were observed between human and mouse ESCs (Cai et al. 2010) as were differences in expression of EO-MES, ARNT and several other genes (Ginis et al.2004).

**Literature references**


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Hox genes encode proteins that contain the DNA-binding homeobox motif and control early patterning of segments in the embryo as well as later events in development (reviewed in Rezsohazy et al. 2015). Mammals have 39 Hox genes arrayed in 4 linear clusters, with each cluster containing 9 to 11 genes. Based on homologies, the genes have been assigned to 13 paralogous groups. The nomenclature of Hox genes uses a letter to indicate the cluster and a number to indicate the paralog group. For example, HOXA4 is the gene in cluster A that is most similar with genes of paralog group 4 from other clusters.

One of the most striking aspects of mammalian Hox gene function is the mechanism of their activation during embryogenesis: the order of genes in a cluster correlates with the timing and location of their activation such that genes at the 3' end of a cluster are activated first and genes at the 5' end of a cluster are activated last. (5' and 3' refer to the transcriptional orientation of the genes in the cluster.) Because development of segments of the embryo proceeds from anterior to posterior this means that the anterior boundaries of expression of 3' genes are more anterior (rostral) and the anterior boundaries of expression of 5' genes are more posterior (caudal).

Expression of HOX genes initiates in the posterior primitive streak at the beginning of gastrulation at approximately E7.5 in mouse. As gastrulation proceeds, further 5' genes are sequentially activated and they too undergo the same chromatin changes and migration. After formation of the axis of the embryo, similar waves of activation of HOXA and HOXD clusters occur in developing limbs beginning at about E9. Retinoids, especially all trans retinoic acid (atRA), participate in initiating the process via retinoid receptors. Other factors such as FGFs and Wnt, also regulate Hox expression. After activation, Hox genes participate in maintaining their own expression (autoregulation), activating later, 5' Hox genes, and repressing prior, 3' Hox genes (crossregulation). Differentiation of embryonal carcinoma cells and embryonic
stem cells in response to retinoic acid is used to model the process in vitro (reviewed in Gudas et al. 2013).

Activation of Hox genes is accompanied by a change from bivalent chromatin to euchromatin (reviewed in Soshnikova and Duboule 2009). Bivalent chromatin has extensive methylation of lysine-9 on histone H3 (H3K9me3), a repressive mark, with interspersed punctate regions of methylation of lysine-4 on histone H3 (H3K4me2, H3K4me3), an activating mark. Euchromatization initiates at the 3' ends of clusters and proceeds towards the 5' ends, with the euchromatin migrating to an active region of the nucleus (reviewed in Montavon and Duboule 2013). This change in chromatin reflects a loss of H3K27me3 and a gain of H3K4me2,3. Polycomb repressive complexes bind H3K27me3 and are responsible for maintenance of repression, KDM6A and KDM6B histone demethylases remove H3K27me3, and members of the trithorax family of histone methylases (KMT2A, KMT2C, KMT2D) methylate H3K4.

**Literature references**


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LGI-ADAM interactions

Location: Developmental Biology

Stable identifier: R-HSA-5682910

Compartments: extracellular region

Synapse formation and maturation require multiple interactions between presynaptic and postsynaptic neurons. These interactions are mediated by a diverse set of synaptogenic proteins (Kegel et al. 2013, Siddiqui & Craig 2011). Initial synapse formation needs both the binding of secreted proteins to presynaptic and postsynaptic receptors, and the direct binding between presynaptic and postsynaptic transmembrane proteins. One class of molecules that plays an important role in cellular interactions in nervous system development and function is the leucine-rich glioma inactivated (LGI) protein family. These are secreted synaptogenic proteins consisting of an LRR (leucine-rich repeat) domain and a epilepsy-associated or EPTP (epitempin) domain (Gu et al. 2002). Both protein domains are generally involved in protein-protein interactions. Genetic and biochemical evidence suggests that the mechanism of action of LGI proteins involves binding to a subset of cell surface receptors belonging to the ADAM (a disintegrin and metalloproteinase) family, i.e. ADAM11, ADAM22 and ADAM23. These interactions play crucial role in the development and function of the vertebrate nervous system mainly mediating synaptic transmission and myelination (Kegel et al. 2013, Novak 2004, Seals & Courtneidge 2003).

Literature references


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Keratins are the major structural protein of vertebrate epidermis, constituting up to 85% of a fully differentiated keratinocyte (Fuchs 1995). Keratins belong to a superfamily of intermediate filament (IF) proteins that form alpha-helical coiled-coil dimers, which associate laterally and end-to-end to form approximately 10 nm diameter filaments. Keratin filaments are heteropolymeric, formed from equal amounts of acidic type I and basic/neutral type 2 keratins. Humans have 54 keratin genes (Schweitzer et al. 2006). They have highly specific expression patterns, related to the epithelial type and stage of differentiation. Roughly half of human keratins are specific to hair follicles (Langbein & Schweizer 2005). Keratin filaments bundle into tonofilaments that span the cytoplasm and bind to desmosomes and other cell membrane structures (Waschke 2008). This reflects their primary function, maintaining the mechanical stability of individual cells and epithelial tissues (Moll et al. 2008).

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Neutrophilic granulocytes (hereafter called granulocytes) are distinguished by multilobulated nuclei and presence of cytoplasmic granules containing antipathogenic proteins (reviewed in Cowland and Borregaard 2016, Yin and Heit 2018). Granulocytes comprise eosinophils, basophils, mast cells, and neutrophils, all of which are ultimately derived from hemopoietic stem cells (HSCs), a self-renewing population of stem cells located in the bone marrow. A portion of HSCs exit self-renewing proliferation and differentiate to form multipotent progenitors (MPPs). MPPs then differentiate to form common myeloid progenitors (CMPs) as well as the erythrocyte lineage. CMPs further differentiate into granulocyte-monocyte progenitors (GMPs) which can then differentiate into monocytes or any of the types of granulocytes (reviewed in Fiedler and Brunner 2012). Granulocytes are the most abundant leukocytes in peripheral blood.

For early granulopoiesis the CEBPA, SPI1 (PU.1), RAR, CBF, and MYB transcription factors are essential. CEBPE, SPI1, SP1, CDP, and HOXA10 transcription factors initiate terminal neutrophil differentiation.

Initially, RUNX1 activates SPI1 (PU.1), which is believed to be the key transcription factor driving the formation of MPPs and CMPs (reviewed in Friedman 2007, Fiedler and Brunner 2012). SPI1, in turn, activates expression of CEBPA, an indispensable transcription factor for granulopoiesis especially important in the transition from CMP to GMP (inferred from mouse homologs in Wilson et al. 2010, Guo et al. 2012, Guo et al. 2014, Cooper et al. 2015). CEBPA, in turn, activates the expression of several transcription factors and receptors characteristic of granulocytes, including CEBPA (autoregulation), CEBPE (Loke et al. 2018, and inferred from mouse homologs in Wang and Friedman 2002, Friedman et al. 2003), GFI1 (inferred from mouse homologs in lidonnici et al. 2010), KLF5 (Federzoni et al. 2014), IL6R (inferred from mouse homologs in Zhang et al. 1998), and CSF3R (Smith et al. 1996). Importantly, CEBPA dimers repress
transcription of MYC (c-Myc) (Johansen et al. 2001, and inferred from mouse homologs in Slomiany et al. 2000, Porse et al. 2001). CEBPA binds CDK2 and CDK4 (Wang et al. 2001) which inhibits their kinase activity by disrupting their association with cyclins thereby limiting proliferation and favoring differentiation of granulocyte progenitors during regular ("steady-state") granulopoiesis (reviewed in Friedman 2015). The transcription factor GFI1 regulates G-CSF signaling and neutrophil development through the Ras activator RasGRP1 (de la Luz Sierra et al. 2010).

Inhibitors of DNA binding (ID) proteins ID1 and ID2 regulate granulopoiesis and eosinophil production such that ID1 induces neutrophil development and inhibits eosinophil differentiation, whereas ID2 induces both eosinophil and neutrophil development (Buitenhuis et al. 2005, Skokowa et al. 2009).

Major infection activates emergency granulopoiesis (reviewed in Manz and Boettcher 2014, Hirai et al. 2015), the production of large numbers of granulocytes in a relatively short period of time. Emergency granulopoiesis is activated by cytokines, CSF2 (GM-CSF) and especially CSF3 (G-CSF, reviewed in Papanopoulos and Watowich 2008, Liongue et al. 2009) which bind receptors, CSF2R and CSF3R, respectively, resulting in expression of CEBPB, which interferes with repression of MYC by CEBPA (inferred from mouse homologs in Zhang et al. 2010) and represses MYC less than CEBPA does (Hirai et al. 2006), leading to proliferation of granulocyte progenitors prior to final differentiation. Both, emergency and steady-state granulopoiesis are regulated by direct interaction of CEBPA (steady-state) or CEBPB (emergency) proteins with NAD+-dependent protein deacetylases, SIRT1 and SIRT2 (Skokowa et al. 2009). G-CSF induces the NAD+-generating enzyme, Nicotinamide phosphoribosyltransferase (NAMPT, or PBEF), that in turn activates sirtuins (Skokowa et al. 2009).

GADD45A and GADD45B proteins are essential for stress-induced granulopoiesis and granulocyte chemotaxis by activation of p38 kinase (Gupta et al. 2006, Salerno et al. 2012). SHP2 is required for induction of CEBPA expression and granulopoiesis in response to CSF3 (G-CSF) or other cytokines independent of SHP2-mediated ERK activation (Zhang et al. 2011).

Transcription of neutrophil granule proteins (e.g. ELANE, MPO, AZU1, DEFA4), that play an essential role in bacterial killing are regulated by CEBPE and SPI1 (PU.1) transcription factors (Gombart et al. 2003, Nakajima et al. 2006). RUNX1 and LEF1 also regulate ELANE (ELA2) mRNA expression by binding to its promotor (Li et al. 2003).

**Literature references**


**Editions**

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</tbody>
</table>
# Table of Contents

- **Introduction** 1
- **Developmental Biology** 2
  - Nervous system development 3
  - Myogenesis 4
  - Regulation of beta-cell development 6
  - Signaling by NODAL 8
  - Transcriptional regulation of white adipocyte differentiation 9
  - Transcriptional regulation of pluripotent stem cells 12
  - Activation of HOX genes during differentiation 14
  - LGI-ADAM interactions 16
  - Keratinization 17
  - Transcriptional regulation of granulopoiesis 18

Table of Contents 20