Published in final edited form as:

Cytokine Growth Factor Rev. 2008 ; 19(0): 357–369. doi:10.1016/j.cytogfr.2008.11.002.

p204, a p200 family protein, as a multifunctional regulator of cell proliferation and differentiation

Yi Luan[a], **Peter Lengyel**[b,*], and **Chuan-Ju Liu**[a,c,**]

[a] Department of Orthopaedic Surgery, New York University School of Medicine, New York, NY 10003, United States

[b] Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar St., New Haven, CT 06520-8024, United States

[c] Department of Cell Biology, New York University School of Medicine, New York, NY 10016, United States

Abstract

The interferon-inducible p200 family comprises a group of homologous mouse and human proteins. Most of these have an N-terminal DAPIN domain and one or two partially conserved, 200 amino acid long C-terminal domains (designated as 200X domain). These proteins play important roles in the regulation of cell proliferation, tissue differentiation, apoptosis and senescence. p200 family proteins are involved also in autoimmunity and the control of tumor growth. These proteins function by binding to various target proteins (e.g. transcription factors, signaling proteins, oncoproteins and tumor suppressor proteins) and modulating target activity. This review concentrates on p204, a murine member of the family and its roles in regulating cell proliferation, cell and tissue differentiation (e.g. of skeletal muscle myotubes, beating cardiac myocytes, osteoblasts, chondrocytes and macrophages) and signaling by Ras proteins. The expression of p204 in various tissues as promoted by tissue-specific transcription factors, its distribution among subcellular compartments, and the controls of these features are also discussed.

Keywords

p204; p200 (Ifi-200/Hin-200) family; Interferon; Cell proliferation; Differentiation

1. Introduction

p204 is a member of the interferon-inducible murine p200 protein family. p202, the earliest member of this family, was identified in 1982 on the basis of the inducibility of its mRNA by interferon. The murine p200 family proteins are encoded by 10 or more genes forming a cluster at the q21–q23 region of chromosome 1 (gene 200 cluster) [1–7]. The murine p200

* Corresponding author at: Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar St., New Haven, CT 06520-8024 United States. Tel.: +1 203 737 2061; fax: +1 203 785 7979. peter.lengyel@yale.edu (P. Lengyel). ** Corresponding author at: Department of Orthopaedic Surgery, New York University School of Medicine, 301 East 17th Street, New York, NY 10003, United States. Tel.: +1 212 598 6103; fax: +1 212 598 6096. chuanju.liu@med.nyu.edu (C.-J. Liu)..

family proteins include the p202a, p202b [8,9], p203 [10,11], p204 [12], p205 (also designated as D3 [13,14]), and p206 proteins [15]. These proteins are encoded among others, by the Ifi202a, Ifi202b, Ifi203, Ifi204 and Ifi205 genes [3]. The homologous (also interferon-inducible) human proteins and the genes encoding them include IFI16 [16,17], MNDA [18,19], AIM2 [20,21] and IFIX [22,23]. The existence of several further predicted p200 proteins encoded by predicted genes was proposed: the murine p207, p208, p209 and p210 proteins encoded by the XP359303, XP484937, NP778191, and XP357160 genes, respectively, and the rat RHIN1, RHIN2 and RHIN3 proteins encoded by the XP222949, XP222950, and XP22952 genes, respectively [24]. Some of the p200 family proteins (e.g. IFI16 [25], IFIX [22] and p203 [11]) exist in several isoforms in consequence of multiple splicing.

The p200 family proteins share a common domain structure [3,24]. With the exception of p202a and p202b, which have a unique N-terminus, all p200 proteins contain a DAPIN domain at their N termini [26,27]. This is followed by either one or two copies of a partially conserved 200 amino acid domain (designated as 200X) at their C termini. The two 200X domains may be adjacent or linked by connecting domains. Particular isoforms of IFIX and of p203 are exceptions, they lack a 200X domain [11,23].

Several studies demonstrated that the p200 family proteins can regulate the proliferation of wild-type and tumor cells [28–32], as well as differentiation [33–42], apoptosis [43–45] and senescence [46,47] and may affect virus proliferation [48]. The possible importance of p200 family proteins in immunomodulation and disease was revealed by the findings that overexpression of p202 can be linked in certain strains of inbred mice to immunological disorders including systemic lupus erythematosus, and that a significant percent of patients with systemic lupus erythematosus or Sjogen's syndrome develop autoantibodies to the p200 family protein IFI16 [49–53].

2. Characteristics of the murine p204 protein

p204 consists of 640 amino acid residues with an apparent molecular weight of 72 kDa [2]. It is encoded by the Ifi204 gene which is part of the gene 200 cluster on murine chromosome 1 [3]. This cluster is located between the erythroid alpha-spectrin and the amyloid P-component loci [3,6]. 204 cDNA (corresponding to the Ifi204 gene) specifies a 2.4 kb mRNA [2].

The N-terminal region (amino acids 1–216) of p204 contains a coiled-coil domain (amino acids 1–88). This domain is designated: as DAPIN and also as PAAD (Pyrin, AIM [54], ASC [54] and DD {Death-domain like}), CARD (caspase-recruitment domain) or PYRIN domain, also abbreviated as PYD. The DAPIN domain is an approximately 90 amino acid motif that has been linked to a larger family of proteins involved, among others, in apoptosis and inflammatory pathways. Functional observations and the 3D domain structure indicate that the DAPIN domain belongs to the death domain family [26,27]. This family also includes the evolutionarily related CARD, DD (death), and DED (death effector) domains, which participate in domain–domain interactions in the larger PYRIN family of proteins [55,56].

The N-terminal region of p204 [3,37] includes a basic amino acid-rich nuclear localization signal (NLS), – LysLysXLysXXLys–, and a canonical leucine-rich nuclear export signal (NES), –LeuXXXLeuLeuXXXLeuXLeu–. In between the NES and the NLS there is a region consisting of four perfect and three imperfect repeats of a seven amino acid long segment. The sequence of a perfect repeat is TSTAQAR. The C-terminal domain of p204 consists of 2, apparently contiguous (200X-type) 200 amino acids domains (1 a type and 1 b type domain) with 34% sequence identity.

The a-type and b-type domains each contain one copy of an MF/LHATVAT/S type sequence which is conserved in all 200 X-type domains [3]. This sequence was reported to be involved in protein/protein interactions. In p204 the a-type domain contains two kinds of pRb-binding motifs: an IXCXE sequence (at position 399–403), and an LXCXE sequence (at position 423–427). The b-type domain contains only a single pRb-binding LXCXE sequence (at position 611–615). The p204 protein amino acid sequence contains potential sites for phosphorylation by various protein kinases including: cAMP-dependent kinase, protein kinase C, calmodulin-dependent protein kinase, MAP kinase, ATM, Cdk2 and casein-kinase 2 [57]. p204 has been shown to be a phosphoprotein [12], and its phosphorylation is apparently involved in the translocation of p204 from the nucleus to the cytoplasm [36,58]. Although it is likely that serine or threonine residues of p204 can be phosphorylated, the site(s) of phosphorylation and the phosphorylating enzyme(s) remain to be identified.

2.1. Regulation of expression in various tissues

p204 was found to be constitutively expressed in the heart, skeletal muscle, kidney, bone marrow, thymus, lymph nodes, spleen and myelomonocytic cells of adult mice, calvaria osteoblasts of neonatal mice as well as in embryonic osteoblasts and hypertrophic chondrocytes [36,37,42,59]. p204 is induced by interferon α, interferon β, interferon γ, and poly(rI:rC) in numerous cultured murine cells [8,12,48,60]. The treatment with interferon increased the level of the p204 protein primarily by increasing the level of 204 mRNA [8,12]. The extent of the induction of p204 by interferons varies among different inbred strains of mice. Thus a comparison of extracts from several interferon-treated cell lines revealed similarly high inducibility (up to 75-fold) of p204 by interferon in BALB/c 3T3, NIH 3T3, and EAT cells but very low inducibility in cell lines originating from C57/BL6 mice [8,12,61].

The levels of p204 (and also of an other p200 family protein, p202) in spleen, thymus, heart, skeletal muscle and lung were compared in adult wild-type C129 mice and C129 mice lacking receptors for α, β and γ interferons. The tissue distributions of p204 (and also of p202) in the above organs were apparently unaffected by the absence of interferon receptors. This indicates that, at least in uninfected mice, the expressions of p204 (and p202) were unaffected by endogenous interferons [62].

p204 was originally isolated as an interferon-inducible protein [5]. However various agents other than interferons can induce p204, and a variety of distinct transcription factors can promote the activation of Ifi204 expression in different tissues (see subsequent sections).

2.2. Localization in various subcellular compartments

Fractionation of cell lysates followed by western blotting and indirect immunofluorescence microscopy revealed that the p204 protein can be nucleolar, nucleoplasmic, cyto plasmic and membrane associated. Its nucleolar localization was confirmed by colocalization with B23 a known nucleolar protein [12].

The subcellular distribution of p204 varied with the cell types and their state of differentiation [34–36,58]. For instance it was predominantly nucleolar in AKR-2B, a cloned murine embryo cell line, mainly nucleoplasmic in an interferon-treated proliferating C2C12 skeletal muscle myoblast line, whereas much of p204 appeared in the cytoplasm during and after the differentiation of C2C12 myoblasts into myotubes. The translocation of p204 from the nucleus to the cytoplasm was correlated with its phosphorylation [36,58]. The various functions of p204 in the different subcellular compartments have been explored (see subsequent sections).

3. Biological functions of the p204 protein

3.1. Regulation of cell proliferation

p204 can inhibit cell proliferation [29,30,32]. p204 from which its b domain was removed lost its antiproliferative activity [60]. However p205, a p200 family protein with only one a domain (and no b domain) has antiproliferative activity revealing that a single 200X domain can make a p200 family protein growth inhibitory [63]. The inhibition of cell proliferation by p204 may occur by multiple mechanisms [30,58,63]. Thus p204 can inhibit the proliferation of a human osteosarcoma cell line (U2OS) with active p53 and pRb, and also of a second osteosarcoma line (Saos2), which is lacking active p53 and pRb [63]. This indicates that the antiproliferative activity of p204 does not have to depend on active p53 or pRb. A difference between the modes of inhibition by p204 of the proliferation of the above two osteosarcoma cell lines was also revealed by the finding that p204 induction resulted in a partial G2/M arrest in Saos2 cultures, but had little effect on cell cycle distribution in U2OS cultures. Furthermore in U2OS cultures, but not in Saos2 cultures, the expression of p204 significantly increased the levels of pRb and its active hypophosphorylated form.

It should be noted that there are other reports according to which p204 inhibits the proliferation of U2OS but not of Saos2 [64]. The basis of this discrepancy among the different reports concerning the problem whether or not pRb is required for the antiproliferative activity of p204 is unclear. The same report which finds that Saos2 proliferation is not inhibited by p204 also describes that p204 inhibits the proliferation of wild-type MEF cells, but not of MEF cells in which the pRb gene was inactivated [64]. Furthermore transfection of p204 in which only the LXCXE sequence in the a domain was mutated seems to inhibit cell proliferation not very differently from wild-type p204 [64]. However p204 from which the C-terminal 72 amino acid segment was deleted in order to remove the pRb-binding sequence (LXCXE) from the b segment, without or together with the mutation of the LXCXE sequence in the a segment was shown not to decrease, but actually to increase cell proliferation as detected by focus forming assays [64]. It remains to be established whether this increase in the rate of cell proliferation is a consequence of the

loss of pRb-binding activity, or of other, as yet uncharacterized consequences of the deletion of the C-terminal segment.

3.1.1. Inhibition of ribosomal RNA synthesis—As noted in the previous section, part of the endogenous p204 may be nucleolar [12]. The nucleolus is the subcellular organ of ribosomal RNA synthesis and ribosome assembly. This consideration together with the antiproliferative activity of p204 and the fact that p202a (another p200 family protein) binds to and inhibits the activity of numerous transcription factors [28,31], prompted the exploration whether p204 inhibits ribosomal RNA synthesis. The experiments revealed that it does [30]. p204 directly associates with the ribosomal RNA-specific UBF-1 transcription factor *in vivo* and *in vitro*. UBF-1 binds strongly to at least two regions of p204: the N-terminal segment linked to the conserved 200 amino acid *a* domain, and the conserved 200 amino acid *b* domain. The inhibition of ribosomal RNA synthesis by p204 may be due to the inhibition by p204 of the specific DNA binding of UBF-1. This was revealed, among others, in magnetic bead binding and footprinting assays [30]. pRb was also shown to inhibit ribosomal RNA synthesis by UBF-1 [65]. It remains to be determined whether there exists an interaction between p204 and pRb in the regulation of ribosomal RNA synthesis. The inhibition of ribosomal RNA synthesis by p204 is likely to contribute to the antiproliferative and differentiation promoting activities of p204.

Sin3a protein, is a corepressor that can be involved in the histone deacetylase (HDAC)-mediated repression of gene expression [66–68]. The binding of Sin3a to p204 was demonstrated by immunochemical copurification followed by mass spectrometry (Liu and Lengyel, unpublished data) and also in a yeast 2 hybrid screen (Caceres and Liu, unpublished data). It remains to be explored whether Sin3a can be involved in the p204-mediated inhibition of gene expression and cell proliferation.

3.2. Regulation of cell differentiation

3.2.1. Role in the differentiation of cultured C2C12 skeletal muscle myoblasts to myotubes—Among 10 adult mouse tissues tested the level of p204 was second highest in skeletal muscle [36]. In cultured C2C12 myoblasts p204 was nucleoplasmic and its level was low. During myoblast fusion this level strongly increased, p204 became phosphorylated (on serine or threonine residues) and the bulk of p204 was translocated from the nucleus to the cytoplasm [36]. This translocation was dependent on the NES present in p204. The increase in p204 level during myoblast fusion was a consequence of MyoD, myogenin, and/or E12/E47 transcription factors binding to several E box sequences in the gene encoding p204, followed by transcription. Overexpression of p204 (in C2C12 myoblasts carrying an inducible p204 expression plasmid) accelerated the fusion of myoblasts to myotubes in (low serum) differentiation medium, and induced the fusion even in (high serum) growth medium. On the other hand, a decrease in the level of p204 in myoblasts carrying an expression plasmid for 204 antisense RNA inhibited the fusion of C2C12 myoblasts to myotubes in differentiation medium. These findings indicate that p204 is required for the differentiation of C2C12 myoblasts [36].

p204 enables this differentiation at least in part by overcoming the inhibition of the activities of the MyoD and E12/E47 transcription factors by the Id (inhibitor of differentiation or of DNA binding) proteins Id1, Id2 and Id3 [38]. These Id proteins were known to inhibit skeletal muscle differentiation by binding and blocking the formation of MyoD and E12/E47 homo and heterodimers, and thereby the specific binding to DNA and the transcriptional activity of MyoD, E12/E47 and other myogenic basic helix– loop–helix (HLH) proteins [69–72]. This in turn inhibits the synthesis of numerous muscle proteins as well as that of p204. The following findings indicate that p204 overcomes the inhibition of myoblast differentiation by Id proteins and also shed some light on the mechanism of p204 action. p204 bound to the Id proteins *in vitro* and *in vivo*. In this binding the *b* domain of p204 and the HLH domain of Id were involved [38]. The latter is also the domain involved in the binding of the Id proteins to MyoD and other bHLH proteins [73]. Addition of p204 overcame the inhibition by the Id proteins of the binding of MyoD and E47 to DNA *in vitro*. A decrease in the p204 level in C2C12 myoblasts by 204 antisense RNA inhibited the MyoD, E47 and other bHLH protein-dependent accumulation, e.g. of the muscle-specific myosin heavy chain protein and also inhibited the fusion of myoblasts to myotubes in differentiation medium [38]. Overexpression of p204 in myoblasts overcame the inhibition by the Id proteins of the MyoD and E47 dependent transcription, and also overcame the inhibition by Id2 of the fusion of myoblasts to myotubes. A decrease in the p204 level in C2C12 myoblasts by antisense RNA increased the level of Id2, whereas an overexpression of ectopic p204 decreased the level of Id proteins even in a culture in growth medium. These and other results revealed that the mechanisms by which p204 counteracts the inhibition of the differentiation by Id proteins include the binding of Id proteins as well as decreasing the levels of these proteins [38]. Further details of these mechanisms are presented in the section concerning the roles of p204 in cardiac myocyte differentiation.

There is a regulatory circuit involving the Id1, Id2, Id3, MyoD and p204 proteins (see Fig. 1A): the Id proteins can bind MyoD (as well as myogenin and E47) and inhibit their various activities. MyoD can transactivate the expression of p204 during myoblast differentiation, and the p204 synthesized can bind the Id proteins and thereby can decrease or prevent the inhibition of MyoD, myogenin and E47 activity by the Id proteins. These interactions among MyoD, p204 and the Id proteins result in a positive feedback loop: p204 whose expression is triggered by MyoD can boost the transcriptional activity of MyoD towards its various target genes including Ifi204, the gene encoding p204 [38].

As noted, the differentiation of cultured myoblasts to myotubes in vitro is triggered by shifting them from high serum medium (GM) to low serum medium (DM). The triggers of myoblast differentiation in vivo have not been identified. The ability of p204 to overcome the Id block of differentiation makes it a possible candidate for serving as a component of the trigger. This is the case especially since overexpression of p204 results in a strong decrease in the Id protein level and it triggers the fusion of cultured myoblasts to myotubes even in high serum growth medium [38].

The level of p202a, another member of the p200 protein family, also increases during myoblast differentiation though only after a delay [74]. The multiple activities of p202a make its role in myoblast fusion incompletely understood. The antiproliferative and

antiapopotic activities of p202a [28,31,32,44,75], together with its inhibition of the transcriptional activity of c-Myc [44] (which in turn may result in a decrease in the expression of Id2) could all support differentiation. Furthermore p202a binds MyoD and inhibits its sequence-specific binding to DNA as well as its synthesis [74]. This might account for the known decrease in the level of MyoD during differentiation. The inhibition of the activity and synthesis of MyoD by p202a might also account for the apparently paradoxical finding that whereas an increase in p204 level prior to induction of differentiation accelerates myoblast differentiation, an increase in the p202a level prior to induction of differentiation inhibits this process [74]. It should be noted that the differentiation of myoblasts to myotubes involves many other participants besides those listed above.

3.2.2. Role in the differentiation of P19 embryonal carcinoma stem cells to beating cardiac myocytes—Among 10 adult mouse tissues tested the level of p204 was the highest in the heart [36]. The p204 level in mouse heart muscle increased during development: it was barely detectable in 10.5-day-old embryos, reached the peak level in 16.5-day-old embryos and remained high until birth and thereafter [36]. Induced expression of p204 was also demonstrated in cultured murine P19 embryonal carcinoma stem cells during their differentiation into beating cardiac myocytes in response to DMSO treatment [34]. Using this model system for myocyte differentiation [76], p204 expression was found to be synergistically transactivated by the cardiac Gata4, Nkx2.5 and Tbx5 transcription factors [34]. The induced expression of p204 was required for the differentiation of P19 cells to cardiac myocytes: DMSO-induced p204 expression in the P19 cells, and ectopic p204 could partially substitute for DMSO in inducing (i) the expression of the Gata4 and Nkx2.5 expression factors and (ii) the differentiation of P19 cells. Moreover ectopic p204 antisense RNA inhibited the differentiation induced by DMSO [34]. The Id proteins (Id1, Id2 and Id3) inhibited the differentiation of P19 cells to myocytes in consequence of the binding of Ids to Gata4 and Nkx2.5 and inhibiting their binding to each other and to DNA, which blocked the transactivation of expression of various cardiac proteins [35]. p204 bound to the Id proteins and increased their nuclear export signal-dependent translocation from the nucleus to the cytoplasm, thereby separating them from the Gata4 and Nkx2.5 nuclear transcription factors (which they inhibited in the nucleus). p204 also accelerated the ubiquitination of Id proteins as well as their degradation by proteasomes (Fig. 1C) [35].

As in the course of skeletal muscle differentiation [38], there also exists a positive feedback loop in cardiac muscle differentiation (Fig. 1A) [35]. This involves p204, Gata4 and Nkx2.5 as well as the Id proteins. p204 expression is activated synergistically by the Gata4, Nkx2.5 and Tbx5 transcription factors, and p204 further enhances the activity of these two factors by overcoming their inhibition by the Id proteins [34,35]. Gata4 is a Zn finger protein [77], Nkx2.5 a homeobox protein [78]. Both of them are novel targets of inhibition by the Id proteins. Fig. 1A shows the analogy between the positive feedback loops functioning in skeletal muscle myoblast and cardiac myocyte differentiation.

An exploration of the embryonic specification of the murine cardiac conduction system revealed (a) the need for Id2 in this process and (b) that Id2 expression was cooperatively transactivated by Nkx2.5 and Tbx5 [72]. Considering that Id proteins were found to inhibit

the differentiation of cardiac muscle myocytes [35] it was proposed that the promotion of Id2 expression may shift the balance of cardiac differentiation from the formation of myocytes toward the formation of conduction system cells [72]. It remains to be established whether or not p204 is involved in the differentiation of this conduction system.

A study of the differentiation of P19CL6 murine embryonal carcinoma stem cells to cardiac myocytes established that the expression of Id2 in the differentiating cells could be transactivated by Gata4 and Nkx2.5 [79].

In view of the fact that Id2 inhibits the activities of Gata4, Nkx2.5 and Tbx5 [34], the findings that Nkx2.5 and Tbx5 promote the expression of Id2 [72] and so do Gata4 and Nkx2.5 [79], suggest the existence of a negative feedback loop in cardiac myocyte differentiation. This is in addition to the positive feedback loop that includes p204 as well as Gata4 and Nkx2.5 (Fig. 1A). The coordination of the activities of two such feedback loops and their biological significance remain to be examined. This examination will have to consider the various effects of p204 on the subcellular location and the degradation of the Id proteins (Fig. 1C).

3.2.3. Role in the differentiation of mesenchymal C2C12 cells to osteoblasts—

p204 is expressed in native osteoblasts and its level is increased in the course of BMP-2-induced osteoblastic differentiation of pluripotent mesenchymal C2C12 cells. The Smad transcription factors downstream from BMP-2 that include Smad1, Smad4 and Smad5, can activate or boost the expression of p204-specific reporter genes. Overexpression of p204 enhances the BMP-2-induced osteoblast differentiation in vitro [37]. Induced expression of p204 in osteogenesis is needed for the Cbfa1 transcription factor-dependent gene activation: suppression of p204 expression by an adenovirus construct encoding antisense 204 RNA abolishes the osteoblast-specific gene activation by Cbfa1 [37]. Bone formation proceeds by the precise coordination of many factors. Deregulation of Cbfa1 leads to metabolic bone disease, including osteoporosis and osteopetrosis [80–82]. p204 associates with and acts as the co-factor of Cbfa1 and enhances the Cbfa1-mediated gene activation and osteogenesis [37] (Fig. 1B). Retinoblastoma protein (Rb) interacts with Cbfa1 and is required for Cbfa1-mediated osteogenesis [83]. In the course of osteogenesis Rb, p204 and Cbfa1 form a ternary protein complex via Rb as a linker [39]. This p204/Rb/Cbfa1 transcription complex is bound to the promoter of the osteocalcin gene. Rb is required for the p204-mediated enhancement of Cbfa1 activity, since mutant p204 lacking one or two Rb-binding motifs fails to enhance Cbfa1-mediated gene activation in C2C12 cells and wild-type p204 does not stimulate Cbfa1-dependent transactivation in the Rb-deficient Saos-2 cell line [39] (Fig. 1B).

Id proteins are also involved in osteogenesis. Ids are upregulated in osteoblast differentiation by BMP-2 signals [84–87] and quickly diminish during the terminal differentiation of osteoblasts triggered by Wnt [88]. Over-expression of Id1, Id2, and Id3 strongly inhibits osteoblast differentiation of C2C12 cells initiated by BMPs [84]. Id proteins, including Id1, Id2 and Id3, associate with Cbfa1 to cause diminished transcription (Fig. 1B) of the alkaline phosphatase (ALP) and osteocalcin (OCL) genes leading to a lower ALP activity and a decrease in OCL production [40]. Id proteins act by inhibiting the sequence-specific binding of Cbfa1 to DNA and by decreasing the expression of Cbfa1 in cells undergoing osteogenic

differentiation. p204 overcomes the Id2-mediated inhibition of Cbfa1-induced ALP activity and OCL production: (1) p204 interferes with the binding of Id2 to Cbfa1 and thereby enables Cbfa1 to bind to the promoters of its target genes and (2) p204 promotes the translocation of Id2 from the nucleus to the cytoplasm, and accelerates the degradation of Id2 by the ubiquitin–proteasome pathway (Fig. 1C) during osteogenesis [40]. Thus p204 handles Id proteins in the same way in this differentiation as in the differentiation of skeletal muscle myotubes and of cardiac myocytes [35,38]. The NES of p204 is required for the enhancement of the translocation to the cytoplasm and the acceleration of the degradation of Id proteins. A p204 mutant lacking NES lost these activities [35,38]. The Cbfa1, p204 and Id proteins are components of a regulatory circuit modulating osteoblast differentiation (Fig. 1B). This differentiation is also regulated by p202, another p200 family protein [89].

3.2.4. Role in the differentiation of pluripotent C3H10T1/2 cells to chondrocytes—p204 is highly expressed in vivo in the differentiating hypertrophic chondrocytes of the tibial growth plate of mouse embryos [42]. Chondrocyte hypertrophy during endochondral ossification is a controlled process in which chondrocytes stop proliferating and differentiate into hypertrophic chondrocytes. This process, which is crucial for the longitudinal growth and development of long bones, requires the action of the Cbfa1 transcription factor [90].

The control of expression and the role of p204 in chondrocyte differentiation were studied in a well established *in vitro* model of chondrogenesis: a micromass culture of pluripotent C3H10T1/2 cells induced to differentiate with BMP2 [91]. In this model system, p204 becomes detectable 7 days after induction at the same time with collagen X, a marker of hypertrophic chondrocytes [42]. The 5′ flanking regulatory region of Ifi204 gene contains consensus binding elements for the transcription factors Cbfa1 and Sox5. Both factors can bind to their respective elements *in vitro* and *in vivo*. The expression of p204 depends on Cbfa1 activity. Sox5 (a transcription factor controlling the differentiation from proliferating to hypertrophic chondrocytes) [92] is able to inhibit the transactivation of the Ifi204 gene by Cbfa1 [42]. Overexpression of p204 accelerates chondrocyte hypertrophy as revealed by the enhanced expression of type X collagen and matrix metalloproteinase 13; whereas knockdown of p204 (by siRNA) abolishes this process. Coimmunoprecipitation assays reveal that p204 associates with Cbfa1 in chondrocyte differentiation. Altered expression of p204 in chondrocyte hypertrophy is also accompanied by altered levels of Indian hedgehog (Ihh) protein and parathyroid hormone/parathyroid hormone related peptide receptor 1 (PTHR1) [93]. Thus, p204 is an important regulator of chondrocyte differentiation (1) acting as a co-activator of Cbfa1 and (2) affecting Ihh/PTHrP signaling (Fig. 2) [42]. This differentiation process is also modulated by the p202 protein, which forms a positive feedback loop with PTHrP [89].

3.2.5. Role in macrophage and lymphocytic differentiation—p204 is induced in peritoneal resident macrophages in mice by poly(rI:rC) treatment *in vivo* and in cultured peritoneal macrophages by IFNs or LPS *in vitro*. These results suggest that p204 might be involved in the differentiation and maturation of the myelomonocytic lineage [59]. The Ifi204 gene is expressed in various mature monocyte/macrophage cells. Furthermore, a

gene-trap approach revealed that the Ifi204 gene is activated by the macrophage colony stimulating factor (M-CSF) in the interleukin-3 (IL-3)-dependent myeloid FD-Fms cell line which differentiates to macrophages in response to M-CSF [33]. p204 expression was found to be transcriptionally activated also by leukemia inhibitory factor (LIF) (as well as by M-CSF) in the M1-Fms myeloid progenitor cell line which differentiates into macrophages in response to LIF or M-CSF [33]. Forced expression of p204 strongly decreased the IL-3- and M-CSF-dependent proliferation of cells from this line, whereas it promoted their M-CSF-induced differentiation to macrophages [33].

The observation that the 204 mRNA level is higher in less mature CD4+CD8+ double-positive thymocytes than in more mature CD4+ or CD8+ single-positive thymocytes [94] suggests that p204 may play a role in lymphocytic differentiation. Notch1, an essential factor for promoting thymocyte maturation, transcriptionally upregulates p204 expression during maturation of CD4+CD8+ double positive thymocytes into both CD4+ and CD8+ single positive lineages [94].

3.3. As a modulator and feedback inhibitor of H-Ras and K-Ras protein activity

Many of the effects of p204 (e.g., the modulation of the activities of the UBF-1 and Cbfa1 transcription factors) are exerted in the nucleolus or the nucleoplasm, respectively [30,37]. However, in the course of the differentiation of various types of cells, part of p204 is translocated from the nucleus to the cytoplasm [34–36]. Thus, e.g., in murine cardiac myocytes and heart slices, p204 is accumulated in the cytoplasm [35].

A yeast-2-hybrid assay, using a cDNA library from murine hearts reveals an interaction between p204 and cytoplasmic Ras protein [58]. This is confirmed by coimmunoprecipitation of p204 with Ras from murine heart extract, and also with endogenous or ectopic H-Ras and K-Ras from cell lysates. Moreover, the binding of purified H-RasGTP (though not H-RasGDP) to purified p204 *in vitro* establishes that the two proteins interact directly. p204 interacts with both wild-type Ras and mutated Ras oncoproteins. p204 inhibits (i) the cleavage of RasGTP to RasGDP by RasGAP, (ii) the binding to RasGTP of the Raf-1, phosphatidylinositol 3-kinase, and Ral-GDS effectors of Ras signaling, and (iii) the promotion by the Ras pathway of the phosphorylation and, thus, activation of downstream targets (e.g., MEK, Akt, and $p38^{MAPK}$) [58].

Oncogenic Ras protein expression triggers the phosphor-ylation and translocation of p204 from the nucleus to the cytoplasm. The resulting localization of both proteins in the same subcellular compartment promotes their interaction. The translocation triggered by Ras oncoprotein can be blocked by the LY294002 inhibitor of phosphatidylinositol 3-kinase. Moreover, Ras does not promote the phosphor-ylation and translocation to the cytoplasm of mutated p204 in which serine 179 was replaced by alanine [58].

p204 overexpression inhibits the anchorage-independent proliferation of cells expressing Ras oncoprotein. Ras oncoprotein triggers (in MEF3T3 cells) the rearrangement of the actin cytoskeleton and the enhancement of cell migration through a membrane. Overexpression of p204 inhibits both of the above [58].

Ras oncoprotein as well as activated wild-type Ras increase the expression of p204 [58]. This is a consequence (at least, in part) of (i) the induction by Ras of Egr-1 transcription factor expression [95,96] and (ii) the induction by Egr-1 of the expression of p204 that is encoded by the Ifi204 gene which contains a binding sequence for Egr-1 [58].

The activation of expression of a single copy of K-Ras oncogene in cultured murine embryonic cells [97,98] suffices to induce the expression of a high level of p204 and its distribution between the nuclei and the cytoplasm [58]. Thus, p204 may serve as a negative feedback inhibitor of Ras activity. It will be of interest to explore the effects of cell-specific conditional inactivation in vivo of the gene encoding p204 on the tumorigenic activity of cells with a single copy of the Ras oncogene.

3.4. p204 and virus replication

Interferon was discovered on the basis of its antiviral activity. p204 is an interferon-inducible protein. These considerations prompted the testing of the effect of p204 on the replication of several interferon-sensitive viruses. For this purpose, a cloned murine embryo cell line was used in which p204 expression was inducible by Muristerone [30]. Under the conditions tested, the induction of p204 did not alter the replication of several viruses, including encephalomyocarditis virus, herpes simplex virus, mouse parainfluenza virus, reovirus, and vesicular stomatitis virus (Liu, C., Compton, S., R., and Lengyel, P., unpublished observations).

It is intriguing that p204 was found to promote the replication of murine cytomegalovirus (CMV) [99]. This conclusion was based on the findings that (1) infection of murine embryo fibroblasts (MEF) with CMV strongly increased p204 expression transcriptionally and (2) transfection of MEFs with plasmids encoding either one of two inactive p204 mutants strongly decreased the yield of CMV. It was proposed that p204 increases CMV replication, at least, in part, by its cell cycle regulatory effect which might account, presumably, for the enhancement by p204 of CMV DNA synthesis [48].

Although, as noted, the interferon-inducible p204 protein promoted CMV replication, nevertheless interferon treatment was found to inhibit the replication of human CMV [100].

4. Summary and perspectives

p204 was originally identified as a member of the interferon-inducible p200 protein family [2]. Later studies revealed that its expression can also be induced by a variety of other extracellular agents in the course of various biological processes (Table 1). The mediators of the induction of several of these agents remain to be identified. The known mediators activating or repressing p204 expression in different tissues in response to various extracellular agents include numerous transcription factors and some repressors (Table 2).

The induction of p204 is a crucial step in the differentiation of various tissues and cells, including skeletal muscle myotubes [36,38], beating cardiac type myocytes [34,35], osteoblasts [37,39,40], chondrocytes [42] (this review Figs. 1 and 2), and macrophages [33].

Future studies on p204 will extend the exploration of its involvement in differentiation to hitherto unexamined tissues.

p204 interacts with numerous proteins (Table 3). Several of these interactions are involved, among others, in the differentiation of various tissues. Thus, the interaction of p204 with the Rb protein results in a complex serving as a coactivator of particular transcription factors (e.g., of Cbfa1 acting in osteoblast differentiation) [39]. The interaction of p204 with the Id proteins, overcomes their inhibition of the differentiation of skeletal muscle myoblasts [38], beating cardiac myocytes [35] and osteoblasts [40]. p204 accomplishes this by sequestering the Id proteins, promoting their translocation from the nucleus to the cytoplasm, and accelerating their degradation by proteasomes [35,38] (Fig. 1C).

The biological significance of the binding of Sin3a [66–68] (a multifunctional agent promoting histone deacetylation by HDAC) to p204, remains to be explored (Liu and Lengyel, unpublished data). Sin3a-mediated repression of gene expression is involved in the control of diverse mammalian developmental pathways.

The interactions of p204 with the UBF-1 protein and the resulting inhibition of ribosomal RNA synthesis [30], as well as with the wild-type Ras protein and the Ras oncoproteins resulting in the inhibition of Ras signaling [58] might promote differentiation by arresting the proliferation of cells and/or affecting their distribution among the various phases of the cell cycle.

The inhibition of Ras oncoprotein signaling by p204 might be part of the defenses against tumorigenesis. The interaction of p204 with Id proteins resulting in the acceleration of their degradation and the inhibition of their activity is also expected to have an antitumorigenic effect [35,38]. This is the case since the increased expression of Id proteins in various human tumors is frequently associated with enhanced malignancy [71,101].

There are numerous observations concerning antitumorigenic activity of p200 family proteins other than p204. p202 is a target of the adenovirus E1A and the SV40 large T oncoproteins [32], it inhibits cMyc activity [44] and has antiproliferative activity on human tumor cells [75,102–104].

Among the human p200 family proteins, AIM2 suppresses mammary tumor growth in a mouse model [20] and the inactivation of the AIM2 gene by genetic and epigenetic mechanisms is frequent in mismatch repair-deficient color-ectal cancers [105]. A second human p200 family protein, IFIX, suppresses breast cancer cell proliferation and tumorigenesis and downregulates HDM2, a principal negative regulator of the p53 tumor suppressor protein [22].

Among the human p200 proteins, it is IFI16 [17] that resembles structurallyp204 the most[3].Increased expression of IFI16 can trigger senescence and permanent cell growth arrest in epithelial cells, and silencing of the IFI16 gene in these cells may contribute to the development of prostate cancer[106]. Overexpression of IFI16 in human osteosarcoma and chondrosarcoma cell lines inhibits cell proliferation and colony formation, and the level of IFI16 is markedly lower in human osteosarcomas than in normal bone [107]. The IFI16 level

inversely correlates with the cancer grade in head and neck squamous cell carcinomas, and ectopic IFI16 reduces both growth rate and invitro transforming activity of a cell line (HNO136) with no detectable expression of IFI16 that was established from a head and neck squamous cell carcinoma tumor [108]. The Pokemon protooncogene [109] inhibits IFI16 expression (Zhang and Liu, unpublished data).

The antitumor activity of the various murine and human p200 proteins is in linewith the assumption that the inhibitions by p204 of wild-type Ras and Ras oncoprotein signaling and Id protein activity are indications of p204s antitumorigenicity and warrants a thorough exploration of this effect.

Probably, the most crucial task in thestudy of the biological functions of p204 is the generation of mice in which the effects of the conditional tissue-specific knockout or overexpression of p204 can be tested in different phases of embryogenesis. The results of such tests might validate and extend (or disprove) conclusions concerning the biological functions of p204 that have been based primarily on *in vitro* studies.

The fact that the p200 gene cluster encodes a set of homo-logous proteins which resemble each other to different extents might complicate the analysis of the effects of gene knockouts by raising problems of redundancy or, at least, partial overlap in the functions of different p200 family proteins. Thus Ifi202a–/– mice (not expressing p202a protein) exhibit the normal phenotype at least under normal unstressed conditions. This is most probably in consequence of the fact that the elimination of p202a expression results in the compensatory overexpression of its close homolog the p202b protein [9].

Acknowledgements

P. Lengyel and C.J. Liu are grateful to their gifted collaborators who made the explorations in their laboratories possible. We apologize to the scientists who made contributions to the field, but have not been cited due to space limitations. Studies in the authors' laboratories were aided by NIH research grants AR050620, AR053210, and AG029388 (to C.J. Liu), AI-12320 (to P. Lengyel) and a grant from the Arthritis National Research Foundation (to C.J. Liu).

Biography

Yi Luan, M.D., Ph.D. received her M.D. degree from Shandong Medical University, P.R. China, in 1994, and her Ph.D. degree in microbiology from Shandong University, P.R. China, in 2007. Since 2005, she has been working on the role of p204 in skeletogenesis in Dr Chuna-ju Liu's Laboratory at New York University School of Medicine.

Peter Lengyel obtained a diploma in Chemical Engineering at the Technical University of Budapest and a Ph.D. in Biochemistry at New York University School of Medicine, as a student of Severo Ochoa. After a postdoctoral year at the Pasteur Institute in Paris with Jacques Monod, he joined the faculty of the Department of Molecular Biophysics and Biochemistry of Yale University in 1965, and is at present Professor Emeritus. His research concerned the decipher ing of the genetic code, the mechanisms of protein biosynthesis, the interferon defense system, and recently, interferon-induced proteins, especially, the p200 family and its roles in the control of transcription, cell proliferation, and differentiation.

Chuan-Ju Liu, Ph.D., holds a dual appointment as associate professor in the Department of Orthopaedic Surgery and the Department of Cell Biology at New York University School of Medicine, New York. He is editor-in-chief of *Open Access Rheumatology Research and Reviews*, managing editor of *Frontiers in Bioscience* and member of the Editorial Boards of several journals, including the International Journal of Biological Chemistry, and Clinical Medicine: Arthritis and Musculoskeletal Disorders. Dr. Liu is recipient of the Harold M. Frost Award from the American Society for Bone and Mineral Research, the Dorothy W. Goldstein Award from the Arthritis Foundation, and the Kappa Delta Award from the American Academy of Orthopaedic Surgeons and the Orthopaedic Research Society.

Abbreviations

AIM2	absent in melanoma 2
ATM	ataxia telangiectasia mutated (protein kinase)
ALP	alkaline phosphatase
BMP-2	bone morpho-genetic protein-2
CARD	caspase recruitment domain
Cbfa1	core-binding factor l

CMV	cytomegalovirus
DAPIN	domain in apoptosis and interferon response
DD	death-domain like
DED	death effector
DMSO	dimethyl sulfoxide
Egr-1	early growth response 1
E1A	early Region 1A
HDAC	histone deacetylase
HLH	helix–loop–helix
Gata4	GATA binding protein 4
HDM2	Mdm2 p53 binding protein homolog
Id	inhibitor of differentiation or of DNA binding
IHH	Indian hedgehog
IFI	interferon-inducible
IFN	interferon
IL-3	interleukin-3
LPS	lipopolysaccharide
LIF	leukemia inhibitor factor
MNDA	myeloid cell nuclear differentiation antigen
MAP	mitogen-activated protein
M-CSF	macrophage colony stimulating factor
MEF	murine embryo fibroblast
NLS	nuclear localization signal
NES	nuclear export signal
OCL	osteocalcin
PAAD	Pyrin AIM ASC and DD
PYD	pyrin domain
Rb	retinoblastoma protein
PTHR1	parathyroid hormone related peptide receptor 1
PTHrP	parathyroid hormone related peptide
siRNA	small interfering RNA
SV40	Simian virus 40

UBF-1 upstream binding protein 1

References

1. Asefa B, Klarmann KD, Copeland NG, Gilbert DJ, Jenkins NA, Keller JR. The interferon-inducible p200 family of proteins: a perspective on their roles in cell cycle regulation and differentiation. Blood Cells Mol Dis. 2004; 32:155–67. [PubMed: 14757431]
2. Choubey D, Snoddy J, Chaturvedi V, Toniato E, Opdenakker G, Thakur A, et al. Interferons as gene activators. Indications for repeated gene duplication during the evolution of a cluster of interferon-activatable genes on murine chromosome 1. J Biol Chem. 1989; 264:17182–9. [PubMed: 2477366]
3. Deschamps S, Meyer J, Chatterjee G, Wang H, Lengyel P, Roe BA. The mouse Ifi200 gene cluster: genomic sequence, analysis, and comparison with the human HIN-200 gene cluster. Genomics. 2003; 82:34–46. [PubMed: 12809674]
4. Lengyel P. From RNase L to the multitalented p200 family proteins: an exploration of the modes of interferon action. J Interferon Cytokine Res. 2008; 28:273–81. [PubMed: 18547157]
5. Lengyel P, Choubey D, Li S- J, Datta B. The interferon-activatable gene 200 cluster: from structure toward function. Semin Virol. 1995; 6:203–13.
6. Opdenakker G, Snoddy J, Choubey D, Toniato E, Pravtcheva D, Seldin MF, et al. Interferons as gene activators: a cluster of six interferon-activatable genes is linked to the erythroid alpha-spectrin locus on murine chromosome 1. Virology. 1989; 171:568–78. [PubMed: 2474894]
7. Samanta, H.; Dougherty, J.; Brawner, ME.; Schmidt, H.; Lengyel, P. Interferon action: cloning of cDNA segments complementary to messenger RNAs induced by interferons.. In: Merigan, T.; Friedman, R.; Fox, CF., editors. Chemistry and Biology of Interferons Relationship to Therapeutics, UCLA Symposium of Molecular and Cellular Biology. Academic Press; New York, NY: 1982. p. 59-72.
8. Choubey D, Lengyel P. Interferon action: cytoplasmic and nuclear localization of the interferon-inducible 52-kD protein that is encoded by the Ifi 200 gene from the gene 200 cluster. J Interferon Res. 1993; 13:43–52. [PubMed: 8454910]
9. Wang H, Chatterjee G, Meyer JJ, Liu CJ, Manjunath NA, Bray-Ward P, et al. Characteristics of three homologous 202 genes (Ifi202a, Ifi202b, and Ifi202c) from the murine interferon-activatable gene 200 cluster. Genomics. 1999; 60:281–94. [PubMed: 10493828]
10. Gribaudo G, Ravaglia S, Guandalini L, Riera L, Gariglio M, Land-olfo S. Molecular cloning and expression of an interferon-inducible protein encoded by gene 203 from the gene 200 cluster. Eur J Biochem. 1997; 249:258–64. [PubMed: 9363777]
11. Zhang Y, Tian Q, Du Y, Cao H, Lengyel P, Kong W. Multiple splicing results in at least two p203 proteins that are expressed in the liver and down-regulated during liver regeneration. Front Biosci. 2008; 13:2444–51. [PubMed: 17981725]
12. Choubey D, Lengyel P. Interferon action: nucleolar and nucleoplasmic localization of the interferon-inducible 72-kD protein that is encoded by the Ifi 204 gene from the gene 200 cluster. J Cell Biol. 1992; 116:1333–41. [PubMed: 1541632]
13. Tannenbaum CS, Major J, Ohmori Y, Hamilton TA. A lipopolysaccharide-inducible macrophage gene (D3) is a new member of an interferon-inducible gene cluster and is selectively expressed in mononuclear phagocytes. J Leukoc Biol. 1993; 53:563–8. [PubMed: 7684766]
14. Weiler SR, Gooya JM, Ortiz M, Tsai S, Collins SJ, Keller JR. D3: a gene induced during myeloid cell differentiation of Linlo c-Kit+ Sca-1(+) progenitor cells. Blood. 1999; 93:527–36. [PubMed: 9885214]
15. Ludlow LE, Purton LE, Klarmann K, Gough DJ, Hii LL, Trapani JA, et al. Cloning and characterisation of Ifi206: a new murine HIN-200 family member. J Cell Biochem. 2008; 103:1270–82. [PubMed: 17786933]
16. Choubey D, Deka R, HoS M. Interferon-inducible IFI16 protein in human cancers and autoimmune diseases. Front Biosci. 2008; 13:598–608. [PubMed: 17981573]

17. Trapani JA, Browne KA, Dawson MJ, Ramsay RG, Eddy RL, Show TB, et al. A novel gene constitutively expressed in human lymphoid cells is inducible with interferon-gamma in myeloid cells. Immunogenetics. 1992; 36:369–76. [PubMed: 1526658]
18. Briggs JA, Burrus GR, Stickney BD, Briggs RC. Cloning and expression of the human myeloid cell nuclear differentiation antigen: regulation by interferon alpha. J Cell Biochem. 1992; 49:82–92. [PubMed: 1644857]
19. Briggs RC, Shults KE, Flye LA, McClintock-Treep SA, Jagasia MH, Goodman SA, et al. Dysregulated human myeloid nuclear differentiation antigen expression in myelodysplastic syndromes: evidence for a role in apoptosis. Cancer Res. 2006; 66:4645–51. [PubMed: 16651415]
20. Chen IF, Ou-Yang F, Hung JY, Liu J, Wang H, Wang SC, et al. AIM2 suppresses human breast cancer cell proliferation in vitro and mam-mary tumor growth in a mouse model. Mol Cancer Ther. 2006; 5:1–7. [PubMed: 16432157]
21. DeYoung KL, Ray ME, Su YA, Anzick SL, Johnstone RW, Trapani JA. Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. Oncogene. 1997; 15:453–7. [PubMed: 9242382]
22. Ding Y, Lee JF, Lu H, Lee MH, Yan DH. Interferon-inducible protein IFIXalpha1 functions as a negative regulator of HDM2. Mol Cell Biol. 2006; 26:1979–96. [PubMed: 16479015]
23. Ding Y, Wang L, Su LK, Frey JA, Shao R, Hunt KK, et al. Antitumor activity of IFIX, a novel interferon-inducible HIN-200 gene, in breast cancer. Oncogene. 2004; 23:4556–66. [PubMed: 15122330]
24. Ludlow LE, Johnstone RW, Clarke CJ. The HIN-200 family: more than interferon-inducible genes? Exp Cell Res. 2005; 308:1–17. [PubMed: 15896773]
25. Johnstone RW, Kershaw MH, Trapani JA. Isotypic variants of the interferon-inducible transcriptional repressor IFI 16 arise through differential mRNA splicing. Biochemistry. 1998; 37:11924–31. [PubMed: 9718316]
26. Pawlowski K, Pio F, Chu Z, Reed JC, Godzik A. PAAD—a new protein domain associated with apoptosis, cancer and autoimmune diseases. Trends Biochem Sci. 2001; 26:85–7. [PubMed: 11166558]
27. Staub E, Dahl E, Rosenthal A. The DAPIN family: a novel domain links apoptotic and interferon response proteins. Trends Biochem Sci. 2001; 26:83–5. [PubMed: 11166557]
28. Choubey D, Li SJ, Datta B, Gutterman JU, Lengyel P. Inhibition of E2F-mediated transcription by p202. EMBO J. 1996; 15:5668–78. [PubMed: 8896460]
29. Lembo M, Sacchi C, Zappador C, Bellomo G, Gaboli M, Pandolfi PP, et al. Inhibition of cell proliferation by the interferon-inducible 204 gene, a member of the Ifi 200 cluster. Oncogene. 1998; 16:1543–51. [PubMed: 9569021]
30. Liu CJ, Wang H, Lengyel P. The interferon-inducible nucleolar p204 protein binds the ribosomal RNA-specific UBF1 transcription factor and inhibits ribosomal RNA transcription. EMBO J. 1999; 18:2845–54. [PubMed: 10329630]
31. Min W, Ghosh S, Lengyel P. The interferon-inducible p202 protein as a modulator of transcription: inhibition of NF-kappa B, c-Fos, and c-Jun activities. Mol Cell Biol. 1996; 16:359–68. [PubMed: 8524315]
32. Xin H, D'Souza S, Fang L, Lengyel P, Choubey D. p202, an interferon-inducible negative regulator of cell growth, is a target of the adenovirus E1A protein. Oncogene. 2001; 20:6828–39. [PubMed: 11687962]
33. Dauffy J, Mouchiroud G, Bourette RP. The interferon-inducible gene, Ifi204, is transcriptionally activated in response to M-CSF, and its expression favors macrophage differentiation in myeloid progenitor cells. J Leukoc Biol. 2006; 79:173–83. [PubMed: 16244109]
34. Ding B, Liu CJ, Huang Y, Hickey RP, Yu J, Kong W, et al. p204 is required for the differentiation of P19 murine embryonal carcinoma cells to beating cardiac myocytes: its expression is activated by the cardiac GATA4, NKX2.5, and TBX5 proteins. J Biol Chem. 2006; 281:14882–9. [PubMed: 16556595]
35. Ding B, Liu CJ, Huang Y, Yu J, Kong W, Lengyel P. p204 protein overcomes the inhibition of the differentiation of P19 murine embryonal carcinoma cells to beating cardiac myocytes by Id proteins. J Biol Chem. 2006; 281:14893–906. [PubMed: 16556596]

36. Liu C, Wang H, Zhao Z, Yu S, Lu YB, Meyer J, et al. MyoD-dependent induction during myoblast differentiation of p204, a protein also inducible by interferon. Mol Cell Biol. 2000; 20:7024–36. [PubMed: 10958697]
37. Liu CJ, Chang E, Yu J, Carlson CS, Prazak L, Yu XP, et al. The interferon-inducible p204 protein acts as a transcriptional coactivator of Cbfa1 and enhances osteoblast differentiation. J Biol Chem. 2005; 280:2788–96. [PubMed: 15557274]
38. Liu CJ, Ding B, Wang H, Lengyel P. The MyoD-inducible p204 protein overcomes the inhibition of myoblast differentiation by Id proteins. Mol Cell Biol. 2002; 22:2893–905. [PubMed: 11940648]
39. Luan Y, Yu XP, Xu K, Ding B, Yu J, Huang Y, et al. The retinoblastoma protein is an essential mediator of osteogenesis that links the p204 protein to the Cbfa1 transcription factor thereby increasing its activity. J Biol Chem. 2007; 282:16860–7. [PubMed: 17439944]
40. Luan Y, Yu XP, Yang N, Frenkel S, Chen L, Liu CJ. p204 protein overcomes the inhibition of Cbfa1-mediated osteogenic differentiation by Id helix–loop–helix proteins. Mol Biol Cell May. 2008; 19:2113–26.
41. Ludlow LE, Purton LE, Klarmann K, Gough DJ, Hii LL, Trapani JA, et al. The role of p202 in regulating hematopoietic cell proliferation and differentiation. J Interferon Cytokine Res. 2008; 28:5–11. [PubMed: 18370867]
42. Zhang Y, Kong L, Carlson CS, Liu CJ. Cbfa1-dependent expression of an interferon-inducible p204 protein is required for chondrocyte differentiation. Cell Death Differ. 2008; 15:1760–71. [PubMed: 18636074]
43. Aglipay JA, Lee SW, Okada S, Fujiuchi N, Ohtsuka T, Kwak JC, et al. A member of the Pyrin family, IFI16, is a novel BRCA1-associated protein involved in the p53-mediated apoptosis pathway. Oncogene. 2003; 22:8931–8. [PubMed: 14654789]
44. Wang H, Ding B, Liu CJ, Ma XY, Deschamps S, Roe BA, et al. The interferon- and differentiation-inducible p202a protein inhibits the transcriptional activity of c-Myc by blocking its association with Max. J Biol Chem. 2000; 275:27377–85. [PubMed: 10835425]
45. Yan DH, Abramian A, Li Z, Ding Y, Wen Y, Liu TJ, et al. P202, an interferon-inducible protein, inhibits E2F1-mediated apoptosis in prostate cancer cells. Biochem Biophys Res Commun. 2003; 303:219–22. [PubMed: 12646190]
46. Xin H, Curry J, Johnstone RW, Nickoloff BJ, Choubey D. Role of IFI 16, a member of the interferon-inducible p200-protein family, in prostate epithelial cellular senescence. Oncogene. 2003; 22:4831–40. [PubMed: 12894224]
47. Xin H, Pereira-Smith OM, Choubey D. Role of IFI 16 in cellular senescence of human fibroblasts. Oncogene. 2004; 23:6209–17. [PubMed: 15208661]
48. Rolle S, De Andrea M, Gioia D, Lembo D, Hertel L, Landolfo S, et al. The interferon-inducible 204 gene is transcriptionally activated by mouse cytomegalovirus and is required for its replication. Virology. 2001; 286:249–55. [PubMed: 11485393]
49. Caposio P, Gugliesi F, Zannetti C, Sponza S, Mondini M, Medico E, et al. A novel role of the interferon-inducible protein IFI16 as inducer of proinflammatory molecules in endothelial cells. J Biol Chem. 2007; 282:33515–29. [PubMed: 17699163]
50. Chen J, Panchanathan R, Choubey D. Stimulation of T cells up-regulates expression of Ifi202, an interferon-inducible lupus susceptibility gene, through activation of JNK/c-Jun pathway. Immunol Lett. 2008; 118:13–20. [PubMed: 18374989]
51. Choubey D, Kotzin BL. Interferon-inducible p202 in the susceptibility to systemic lupus. Front Biosci. 2002; 7:e252–262. [PubMed: 11991834]
52. Mondini M, Vidali M, De Andrea M, Azzimonti B, Airo P, D'Ambrosio R, et al. A novel autoantigen to differentiate limited cutaneous systemic sclerosis from diffuse cutaneous systemic sclerosis: the interferon-inducible gene IFI16. Arthritis Rheum. 2006; 54:3939–44. [PubMed: 17133607]
53. Rozzo SJ, Allard JD, Choubey D, Vyse TJ, Izui S, Peltz G, et al. Evidence for an interferon-inducible gene, Ifi202, in the susceptibility to systemic lupus. Immunity. 2001; 15:435–43. [PubMed: 11567633]

54. Stehlik C, Krajewska M, Welsh K, Krajewski S, Godzik A, Reed JC. The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor-kappa B and pro-caspase-1 regulation. Biochem J. 2003; 373:101–13. [PubMed: 12656673]
55. Stehlik C, Fiorentino L, Dorfleutner A, Bruey JM, Ariza EM, Sagara J, et al. The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in nuclear factor kappaB activation pathways. J Exp Med. 2002; 196:1605–15. [PubMed: 12486103]
56. Stehlik C, Reed JC. The PYRIN connection: novel players in innate immunity and inflammation. J Exp Med. 2004; 200:551–8. [PubMed: 15353551]
57. Pinna LA, Ruzzene M. How do protein kinases recognize their substrates? Biochim Biophys Acta. 1996; 1314:191–225. [PubMed: 8982275]
58. Ding B, Lengyel P. p204 protein is a novel modulator of Ras activity. J Biol Chem. 2008; 283:5831–48. [PubMed: 18165224]
59. Gariglio M, De Andrea M, Lembo M, Ravotto M, Zappador C, Valente G, et al. The murine homolog of the HIN 200 family, Ifi 204, is constitutively expressed in myeloid cells and selectively induced in the monocyte/macrophage lineage. J Leukoc Biol. 1998; 64:608–14. [PubMed: 9823765]
60. Gribaudo G, Riera L, De Andrea M, Landolfo S. The antiproliferative activity of the murine interferon-inducible Ifi 200 proteins depends on the presence of two 200 amino acid domains. FEBS Lett. 1999; 456:31–6. [PubMed: 10452524]
61. Gariglio M, Panico S, Cavallo G, Divaker C, Lengyel P, Landolfo S. Impaired transcription of the poly rI:rC- and interferon-activatable 202 gene in mice and cell lines from the C57BL/6 strain. Virology. 1992; 187:115–23. [PubMed: 1531277]
62. Wang H, Ding B, Liu CJ, Ma XY, Deschamps S, Roe BA, et al. The increase in levels of interferon-inducible proteins p202a and p202b and RNA-dependent protein kinase (PKR) during myoblast differentiation is due to transactivation by MyoD: their tissue distribution in uninfected mice does not depend on interferons. J Interferon Cytokine Res. 2002; 22:729–37. [PubMed: 12162885]
63. Asefa B, Dermott JM, Kaldis P, Stefanisko K, Garfinkel DJ, Keller JR. p205, a potential tumor suppressor, inhibits cell proliferationvia multiple pathways of cell cycle regulation. FEBS Lett. 2006; 580:1205–14. [PubMed: 16458891]
64. Hertel L, Rolle S, De Andrea M, Azzimonti B, Osello R, Gribaudo G, et al. The retinoblastoma protein is an essential mediator that links the interferon-inducible 204 gene to cell-cycle regulation. Oncogene. 2000; 19:3598–608. [PubMed: 10951565]
65. Cavanaugh AH, Hempel WM, Taylor LJ, Rogalsky V, Todorov G, Rothblum LI. Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. Nature. 1995; 374:177–80. [PubMed: 7877691]
66. Alland L, Muhle R, Hou H, Potes J, Chin L, Schreiber-Agus N, et al. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature. 1997; 387:49–55. [PubMed: 9139821]
67. Ayer DE, Laherty CD, Lawrence QA, Armstrong AP, Eisenman RN. Mad proteins contain a dominant transcription repression domain. Mol Cell Biol. 1996; 16:5772–81. [PubMed: 8816491]
68. Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE. Histone deacetylase activity is required for full transcriptional repression by mSin3A. Cell. 1997; 89:341–7. [PubMed: 9150133]
69. Benezra R, Davis RL, Lockshon D, Turner DL, Weintraub H. The protein Id: a negative regulator of helix–loop–helix DNA binding proteins. Cell. 1990; 61:49–59. [PubMed: 2156629]
70. Gupta GP, Perk J, Acharyya S, de Candia P, Mittal V, Todorova-Manova K, et al. ID genes mediate tumor reinitiation during breast cancer lung metastasis. Proc Natl Acad Sci USA. 2007; 104:19506–11. [PubMed: 18048329]
71. Iavarone A, Lasorella A. ID proteins as targets in cancer and tools in neurobiology. Trends Mol Med. 2006; 12:588–94. [PubMed: 17071138]
72. Moskowitz IP, Kim JB, Moore ML, Wolf CM, Peterson MA, Shendure J, et al. A molecular pathway including Id2, Tbx5, and Nkx2-5 required for cardiac conduction system development. Cell. 2007; 129:1365–76. [PubMed: 17604724]

73. Norton JD, Deed RW, Craggs G, Sablitzky F. Id helix–loop–helix proteins in cell growth and differentiation. Trends Cell Biol. 1998; 8:58–65. [PubMed: 9695810]
74. Datta B, Min W, Burma S, Lengyel P. Increase in p202 expression during skeletal muscle differentiation: inhibition of MyoD protein expression and activity by p202. Mol Cell Biol. 1998; 18:1074–83. [PubMed: 9448005]
75. Yan DH, Wen Y, Spohn B, Choubey D, Gutterman JU, Hung MC. Reduced growth rate and transformation phenotype of the prostate cancer cells by an interferon-inducible protein, p202. Oncogene. 1999; 18:807–11. [PubMed: 9989832]
76. Rudnicki MA, Jackowski G, Saggin L, McBurney MW. Actin and myosin expression during development of cardiac muscle from cultured embryonal carcinoma cells. Dev Biol. 1990; 138:348–58. [PubMed: 2318340]
77. Molkentin JD. The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. J Biol Chem. 2000; 275:38949–52. [PubMed: 11042222]
78. Harvey RP. NK-2 homeobox genes and heart development. Dev Biol. 1996; 178:203–16. [PubMed: 8812123]
79. Lim JY, Kim WH, Kim J, Park SI. Induction of Id2 expression by cardiac transcription factors GATA4 and Nkx2.5. J Cell Biochem. 2008; 103:182–94. [PubMed: 17559079]
80. Marzia M, Sims NA, Voit S, Migliaccio S, Taranta A, Bernardini S, et al. Decreased c-Src expression enhances osteoblast differentiation and bone formation. J Cell Biol. 2000; 151:311–20. [PubMed: 11038178]
81. Miura Y, Miura M, Gronthos S, Allen MR, Cao C, Uveges TE, et al. Defective osteogenesis ofthestromal stemcells predisposes CD18-null mice to osteoporosis. Proc Natl Acad Sci USA. 2005; 102:14022–7. [PubMed: 16172402]
82. Quack I, Vonderstrass B, Stock M, Aylsworth AS, Becker A, Brueton L, et al. Mutation analysis of core binding factor A1 in patients with cleidocranial dysplasia. Am J Hum Genet. 1999; 65:1268–78. [PubMed: 10521292]
83. Thomas DM, Carty SA, Piscopo DM, Lee JS, Wang WF, Forrester WC, et al. The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. Mol Cell. 2001; 8:303–16. [PubMed: 11545733]
84. Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. J Cell Biol. 1994; 127:1755–66. [PubMed: 7798324]
85. Ogata T, Wozney JM, Benezra R, Noda M. Bonemorphogeneticprotein 2 transiently enhances expression of a gene, Id (inhibitor of differentiation), encoding a helix–loop–helix molecule in osteoblast-like cells. Proc Natl Acad Sci USA. 1993; 90:9219–22. [PubMed: 8415680]
86. Pache G, Schafer C, Wiesemann S, Springer E, Liebau M, Reinhardt HC, et al. Up-regulation of Id-1 via BMP-2 receptors induces reactive oxygen species in podocytes. Am J Physiol Renal Physiol. 2006; 291:F654–62. [PubMed: 16622178]
87. Vinals F, Reiriz J, Ambrosio S, Bartrons R, Rosa JL, Ventura F. BMP-2 decreases Mash1 stability by increasing Id1 expression. EMBO J. 2004; 23:3527–37. [PubMed: 15318167]
88. Peng Y, Kang Q, Luo Q, Jiang W, Si W, Liu BA, et al. Inhibitor of DNA binding/differentiation helix–loop–helix proteins mediate bone morphogenetic protein-induced osteoblast differentiation of mesenchymal stem cells. J Biol Chem. 2004; 279:32941–9. [PubMed: 15161906]
89. Kong L, Liu CJ. Mediation of chondrogenic and osteogenic differentiation by an interferon-inducible p202 protein. Cell Mol Life Sci. 2008; 65:3494–506. [PubMed: 18791844]
90. Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. Dev Cell. 2002; 2:389–406. [PubMed: 11970890]
91. Atkinson BL, Fantle KS, Benedict JJ, Huffer WE, Gutierrez-Hart-mann A. Combination of osteoinductive bone proteins differentiates mesenchymal C3H/10T1/2 cells specifically to the cartilage lineage. J Cell Biochem. 1997; 65:325–39. [PubMed: 9138089]
92. Lefebvre V, Li P, de Crombrugghe B. A new long form of Sox5 (LSox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J. 1998; 17:5718–33. [PubMed: 9755172]

93. Kronenberg HM. PTHrP and skeletal development. Ann N Y Acad Sci. 2006; 1068:1–13. [PubMed: 16831900]

94. Deftos ML, Huang E, Ojala EW, Forbush KA, Bevan MJ. Notch1 signaling promotes the maturation of CD4 and CD8 SP thymocytes. Immunity. 2000; 13:73–84. [PubMed: 10933396]

95. McMahon SB, Monroe JG. Activation of the p21ras pathway couples antigen receptor stimulation to induction of the primary response gene egr-1 in B lymphocytes. J Exp Med. 1995; 181:417–22. [PubMed: 7807022]

96. Thiel G, Cibelli G. Regulation of life and death by the zinc finger transcription factor Egr-1. J Cell Physiol. 2002; 193:287–92. [PubMed: 12384981]

97. Bardeesy N, Sinha M, Hezel AF, Signoretti S, Hathaway NA, Sharpless NE, et al. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. Nature. 2002; 419:162–7. [PubMed: 12226664]

98. Tuveson DA, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell. 2004; 5:375–87. [PubMed: 15093544]

99. Hertel L, De Andrea M, Azzimonti B, Rolle A, Gariglio M, Landolfo S. Theinterferon-inducible204gene, amemberofthe Ifi200family, isnot involved in the antiviral state induction by IFN-alpha, but is required by the mouse cytomegalovirus for its replication. Virology. 1999; 262:1–8. [PubMed: 10489335]

100. Schneider K, Loewendorf A, De Trez C, Fulton J, Rhode A, Shumway H, et al. Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus. Cell Host Microbe. 2008; 3:67–76. [PubMed: 18312841]

101. Suh HC, Leeanansaksiri W, Klarmann KD, Renn K, Gooya J, et al. Id1 immortalizes hematopoietic progenitors in vitro and promotes a myeloproliferative disease in vivo. Oncogene. 2008; 27:5612–23. [PubMed: 18542061]

102. Wen Y, Giri D, Yan DH, Spohn B, Zinner RG, Xia W, et al. Prostate-specific antitumor activity by probasin promoter-directed p202 expression. Mol Carcinog. 2003; 37:130–7. [PubMed: 12884364]

103. Wen Y, Yan DH, Spohn B, Deng J, Lin SY, Hung MC. Tumor suppression and sensitization to tumor necrosis factor alpha-induced apoptosis by an interferon-inducible protein, p202, in breast cancer cells. Cancer Res. 2000; 60:42–6. [PubMed: 10646849]

104. Wen Y, Yan DH, Wang B, Spohn B, Ding Y, Shao R, et al. p202, an interferon-inducible protein, mediates multiple antitumor activities in human pancreaticcancerxenograft models. Cancer Res. 2001; 61:7142–7. [PubMed: 11585747]

105. Woerner SM, Kloor M, Schwitalle Y, Youmans H, Doeberitz MK, Gebert J, et al. The putative tumor suppressor AIM2 is frequently affected by different genetic alterations in microsatellite unstable colon cancers. Genes Chromosomes Cancer. 2007; 46:1080–9. [PubMed: 17726700]

106. Alimirah F, Chen J, Xin H, Choubey D. Androgen receptor auto-regulates its expression by a negative feedback loop through upregulation of IFI16 protein. FEBS Lett. 2006; 580:1659–64. [PubMed: 16494870]

107. Zhang Y, Howell RD, Alfonso DT, Yu J, Kong L, Wittig JC, et al. IFI16 inhibits tumorigenicity and cell proliferation of bone and cartilage tumor cells. Front Biosci. 2007; 12:4855–63. [PubMed: 17569615]

108. De Andrea M, Gioia D, Mondini M, Azzimonti B, Reno F, Pecorari G, et al. Effects of IFI16 overexpression on the growth and doxorubicin sensitivity of head and neck squamous cell carcinoma-derived cell lines. Head Neck. 2007; 29:835–44. [PubMed: 17510972]

109. Maeda T, Hobbs RM, Merghoub T, Guernah I, Zelent A, Cordon-Cardo C, et al. Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. Nature. 2005; 433:278–85. [PubMed: 15662416]

110. Sun XH, Copeland NG, Jenkins NA, Baltimore D. Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix–loop–helix proteins. Mol Cell Biol. 1991; 11:5603–11. [PubMed: 1922066]

111. Guo J, Chung UI, Yang D, Karsenty G, Bringhurst FR, Kronenberg HM. PTH/PTHrP receptor delays chondrocyte hypertrophy via both Runx2-dependent and -independent pathways. Dev Biol. 2006; 292:116–28. [PubMed: 16476422]

112. Kronenberg HM. The role of the perichondrium in fetal bone development. Ann NY Acad Sci. 2007; 1116:59–64. [PubMed: 18083921]

113. Gribaudo G, Riera L, Hertel L, Landolfo S. In vitro and in vivo expression analysis of the interferon-inducible 203 gene. J Interferon Cytokine Res. 1999; 19:129–36. [PubMed: 10090398]

114. Sen GC, Sarkar SN. Transcriptional signaling by double-stranded RNA: role of TLR3. Cytokine Growth Factor Rev. 2005; 16:1–14. [PubMed: 15733829]

115. De Andrea M, Zannetti C, Noris E, Gariglio M, Azzimonti B, Landolfo S. The mouse interferon-inducible gene Ifi204 product interacts with the Tpr protein, a component of the nuclear pore complex. J Interferon Cytokine Res. 2002; 22:1113–21. [PubMed: 12513910]

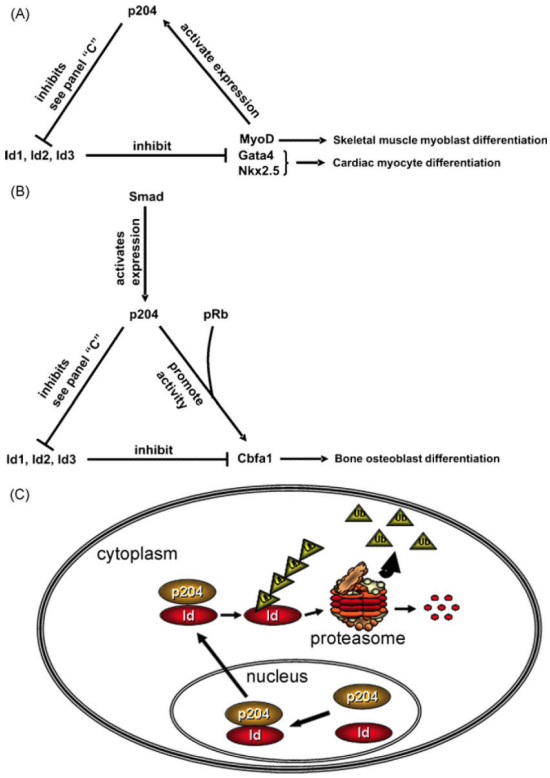

Fig. 1.

Schematic representation of the roles of p204 in overcoming the inhibition by Id proteins of differentiation of skeletal muscle myoblasts, cardiac myocytes, and bone osteoblasts. (A) Regulatory circuit involving p204, Id1, Id2, Id3, and MyoD or GATA4 and Nkx2.5 proteins in skeletal muscle or cardiac myocyte differentiation, respectively. In myoblast differentiation, myogenin can substitute for MyoD in transactivating the gene encoding p204 [36]. Id proteins bind and inhibit the activities of MyoD and myogenin. They also inhibit the activities of MyoD and myogenin indirectly by binding the E12 and E47 proteins with which MyoD or myogenin form heterodimers [110]. The regulatory circuit involved in skeletal muscle myoblast differentiation functions as a positive feedback loop (see Section 3.2.1 and [38]). In the regulatory circuit involved in cardiac myocytes differentiation there also exists a positive feedback loop (see Section 3.2.2 and [35]). Moreover, recent findings reveal that Nkx2.5 and GATA4 [72], and also Nkx2.5 and Tbx5 [79] can also promote Id2 expression, suggesting the existence also of a negative feedback loop which is not shown in A (see however Section 3.2.2). (B) Regulatory circuit involving p204, Id1, Id2, Id3, and Cbfa1 in bone osteoblast differentiation. In this process, a Smad transcription factor complex transactivates the transcription of Smad target genes, including the gene encoding p204. Serving as a bridge, pRb links p204 and Cbfa1. In this ternary complex both p204 and pRb are activating cofactors of Cbfa1. Id proteins bind Cbfa1 and inhibit its binding to its target genes. p204 overcomes this inhibition. (C) Diagram of the process by which p204 overcomes the inhibitions by Id proteins of the differentiation of myoblasts, myocytes, and osteoblasts. p204 binds Id proteins in the nucleus and the resulting complex is translocated to the cytoplasm. In the cytoplasm, p204 promotes the ubiquitination of the Id proteins. The ubiquitinated Id proteins are degraded by proteasomes. Ub, ubiquitin.

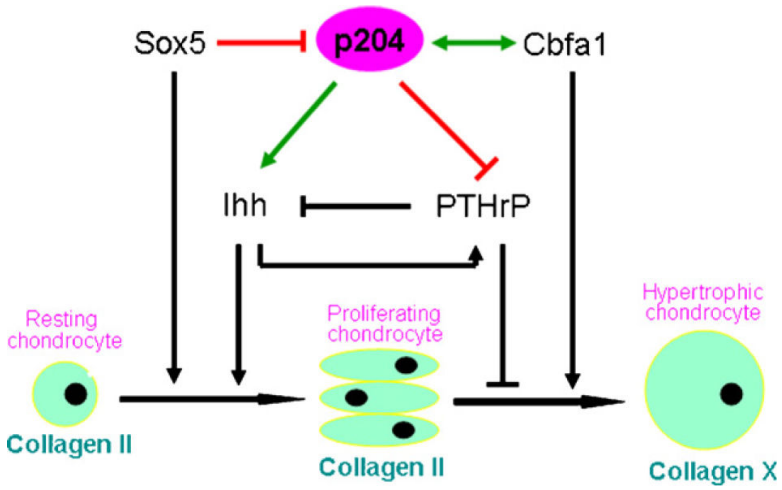

Fig. 2.

Proposed model for the various activities of p204 in chondrocyte differentiation. In differentiating chondrocytes, the Cbfa1 transcription factor promotes and the Sox5 transcription factor inhibits the expression of the gene encoding p204. p204, which associates with Cbfa1, serves as a required cofactor of Cbfa1 in promoting hypertrophic chondrocyte differentiation. Chondrocytes go through a process of proliferation and then further differentiate into hypertrophic chondrocytes. PTHrP keeps chondrocytes proliferating and delays their further differentiation. Ihh increases the synthesis of PTHrP and promotes the differentiation of proliferative chondrocytes [111,112]. Thus, Ihh and PTHrP establish a negative feedback loop that regulates hypertrophic chondrocyte differentiation. p204 regulates chondrocyte hypertrophy by modulating PTHrP/Ihh signaling: it stimulates the expression of Ihh whereas it represses PTHrP expression.

Table 1

Agents inducing the expression of p204 in various cells and/or tissues and mediators involved in the induction.

Agents	Cells and tissues	Reference
Interferons	In various cultured cells. The presence of GA boxes in the Ifi204 gene makes it conceivable that IRF proteins may be involved in p204 induction by interferons	[2]
BMP2, BMP4	In osteoblast differentiation. Mediators can be Smads, possibly, together with Cbfa1. In chondrocyte differentiation, mediators include Cbfa1	[37,42]
LIF	In macrophage differentiation, mediators not identified	[33]
M-CSF	In macrophage differentiation, mediators not identified	[33]
Notch	In T cell differentiation, mediators not Identified	[94]
CM virus	Mediators in cells infected with CM virus not identified	[48]
LPS		[59]
DMSO	E.g., in P19 murine embryonal carcinoma stem cells. Mediators probably include Gata4, Nkx2.5, and Tbx5	[34]
dsRNA, polyI.polyC	In mice injected with dsRNA or polyI.polyC Toll-like receptors and induced interferons may be among the mediators	[113,114]

Table 2

Transcription regulators activating or repressing p204 expression in various murine cells or tissues.

Regulators	Murine cells and tissues	Reference
MyoD, myogenin, E47 (Tf)	C2C12 thigh muscle myoblasts differentiating to myotubes, 10T1/2 fibroblasts	[36]
Gata4, Nkx2.5, Tbx5 (Tf)	P19 embryonal carcinoma stem cells differentiating to cardiac type beating myocytes, neonatal rat cardiac myocytes, murine embryonic fibroblasts	[34]
Smad1, Smad4, Smad5 (Tf)	C2C12 pluripotent mesenchymal cells differentiating to osteoblasts	[37]
Cbfa1 (Tf), Sox5 (Rp)	Pluripotent C3H 10T1/2 cells differentiating to chondrocytes	[42]
Egr-1 (Tf)	Egr-1 expression is triggered by wild-type or oncogenic H-RasGTP or K-RasGTP complex in 3T3 cells and in cultured murine embryonic cells	[58] [95,96]

Tf, transcription factor; Rp, repressor.

Table 3

p204-interacting proteins.

Proteins bound by p204	Effects of the binding of p204	Reference
UBF-1 ribosomal RNA transcription factor	Inhibition of the binding of UBF-1 to DNA and of ribosomal RNA transcription	[30]
pRb, p107, p130 pocket proteins	p204-pRb complex is a coactivator of the Cbfal transcription factor. Effects on cell cycling remain to be explored	[39,42]
Id1, Id2, Id3 inhibitors of differentiation and of DNA binding	p204 sequesters the Id proteins, promotes their translocation from the nucleus to the cytoplasm, and their ubiquitination and degradation by proteasomes. Thereby, p204 overcomes the inhibition by Ids of their target proteins	[35,38,40]
Tpr nuclear pore component	Binding of Tpr is likely to facilitate the translocation of p204 between the nucleus and the cytoplasm	[115]
H-Ras and K-Ras signaling proteins; their mutants include oncoproteins	p204 binding to the H-RasGTP and K-RasGTP (wild-type or oncoprotein) complexes inhibits the binding to the complexes of several Ras mediators and, thereby, blocks signaling	[58]
Sin3a involved in promoting histone deacetylation by HDAC	Effect of p204 binding remains to be explored	Liu and Lengyel (unpublished data)