Homodimeric and Heterodimeric Interactions among Vertebrate Basic Helix–Loop–Helix Transcription Factors

Ana Lilia Torres-Machorro

Laboratorio de Biología Celular, Departamento de Investigación en Fibrosis Pulmonar, Instituto Nacional de Enfermedades Respiratorias “Ismael Cosío Villegas”, Tlalpan, Mexico City 14080, Mexico; ana.torres@iner.gob.mx; Tel.: +52-(55)-54871700 (ext. 9257)

Abstract: The basic helix–loop–helix transcription factor (bHLH TF) family is involved in tissue development, cell differentiation, and disease. These factors have transcriptionally positive, negative, and inactive functions by combining dimeric interactions among family members. The best known bHLH TFs are the E-protein homodimers and heterodimers with the tissue-specific TFs or ID proteins. These cooperative and dynamic interactions result in a complex transcriptional network that helps define the cell’s fate. Here, the reported dimeric interactions of 67 vertebrate bHLH TFs with other family members are summarized in tables, including specifications of the experimental techniques that defined the dimers. The compilation of these extensive data underscores homodimers of tissue-specific bHLH TFs as a central part of the bHLH regulatory network, with relevant positive and negative transcriptional regulatory roles. Furthermore, some sequence-specific TFs can also form transcriptionally inactive heterodimers with each other. The function, classification, and developmental role for all vertebrate bHLH TFs in four major classes are detailed.

Keywords: transcriptional regulation; E-proteins; ID proteins; sequence-specific transcription factors; DNA binding; Class II bHLH transcription factors; protein–protein interactions

1. Introduction

Transcription factors (TFs) are proteins that are directly involved in the activation or repression of RNA synthesis from a DNA template [1], most of the time by recognizing specific DNA sequences [2]. Thus, a set of related sequences preferred by a given transcription factor are known as the TFs’ DNA-binding motifs [2].

TFs can be broadly classified as either basal (general) or sequence-specific TFs [3]. The general TFs recognize the core promoter and are directly involved in RNA polymerase recruitment and transcription initiation. In contrast, the sequence-specific TFs regulate transcription initiation at specific promoters by identifying precise DNA motifs located in enhancers. These enhancers can be proximal or distal to the core promoter [3]. The signaling between the sequence-specific transcription factors and the core machinery is mediated by co-activators and co-repressors [3].

The sequence-specific DNA-binding TFs have also been classified based on their well-defined DNA-binding protein domains [4]. These TF families include the basic helix-loop–helix (bHLH), C2H2 zinc finger (ZF), homeodomain, and basic leucine zipper (LZ) groups (reviewed in [2]).

TFs usually cooperate and synergize with other TFs through extensive protein–protein interactions within their TF family and different families [5]. This combinatorial TF structure provides precision and flexibility to the transcriptional program operating in diverse cell types and tissues [5]. However, a detailed view of how specialized transcriptional networks function is still an emerging research field, despite the enormous progress.

The latest, 2019, bHLH TF family comprehensive review [6] summarized their role in various regulatory pathways. However, in most cases, it did not detail the dimeric...
form involved. This work aims to summarize the complexity of the vertebrate bHLH TFs network, recapitulating all protein–protein interactions known among E-protein interacting bHLH TFs. In addition, detailed information about the experimental approaches defining these interactions is included. Tissue-specific TFs dimers with E-proteins are extensively known. The new conceptual insight from this review highlights the regulatory relevance of tissue-specific dimers not involving E-proteins, reshaping the information summarized before [6] and expanding the classical model.

2. The bHLH TF Family

The bHLH family is one of the most prominent among transcription factors [1,5] and is involved in cell differentiation and tissue development [7,8] (Figure 1). Table S1 lists the developmental involvement of the TFs summarized in this review. These TFs share a characteristic protein structure composed of a basic region [9] that interacts with DNA and a neighboring helix–loop–helix region that mediates dimerization [10]. Most bHLH dimers recognize the E-box, a hexameric sequence in the DNA with the consensus sequence CANNTG [11]. Nevertheless, further characterization and classification of the bHLH TFs groups revealed that some bHLH TFs could also recognize alternative sequences such as the N-box and the ESE-box [12,13]. These bHLH TFs modulate gene expression through dimer formation, combining activators or repressors with ubiquitous proteins (E-proteins) [7,8].

![Figure 1](image_url)

**Figure 1.** Developmental involvement of bHLH TFs. The diagram shows some developmental pathways regulated in part by bHLH TFs. Examples of specific dimeric bHLH TFs forms involved in differentiation and tissue/cell development are shown. Protein dimers are written as monomers separated by a diagonal. The bHLH TFs also have a solid contribution to disease, which is thoroughly reviewed elsewhere [14–17].

3. Classifications of the bHLH TFs

Back in the 1990s, the bHLH TFs from *Drosophila* and mammals were initially classified into three groups: Class A, B, and ID [18]. Class A corresponded to proteins expressed in all tissues tested and included E12 and E47. MyoD belonged to the Class B group characterized by TFs expressed only in some tissues as heterodimers [19]. The ID class opposed the action of A and B TFs [20].
With the burgeoning number of bHLH TFs being identified, several other classification schemes have emerged to accommodate our growing understanding. The Murre Lab initially classified the bHLH TFs into six classes (I–VI, Table 1) based upon dimerization capabilities, DNA-binding specificities, and tissue distribution [6,21,22]. The Class I group, or E-proteins, were expressed in many tissues [23] and could participate in homo- and heterodimers. E-proteins were initially considered to have redundant roles with other E-proteins [24,25]. However, dimerization partners usually have a preferred E-protein [25–35]. MYOD1, NEUROD1, and SCX belonged to the Class II group, characterized by tissue-specific expression and heterodimerization with E-proteins. Class II was extended in 2002 when bHLH TFs were reviewed again, and new TFs were added after identification with a computational approach [36]. Class III TFs had an additional leucine zipper (LZ) motif, with the TF MYC as an example. Proteins heterodimerizing with class III TFs (MAD, MAX, MXI) belonged to the Class IV group. The ID proteins, which lacked the basic domain and interacted with Class I and II proteins to repress their function, constitute the Class V group. The Class VI TFs were homologous to Drosophila’s bHLH TFs hairy and enhancer of split and generally functioned as repressors (e.g., HES1, HEY2). Later, Class VII was added to accommodate the PAS-domain proteins [6].

Table 1. bHLH TFs classification used in this work.

| bHLH Class | Characteristic | Homodimerization | Heterodimerization | Examples | Activity | PDB ID * |
|------------|---------------|------------------|--------------------|----------|---------|---------|
| I E proteins | Yes | Classes I, II, V, and VI TFs | TCF3, TCF4 | A | 6OD4 |
| II Tissue specific | Yes | Classes I, II, V, and VI TFs | NEUROD1, TWIST1 | A or R | 2QL2 |
| III LZ domain | * | Classes III and IV | MYC, SRBEF1 | A or R | 2A93 |
| IV LZ domain | * | Classes III and IV | MAD, MAX | A or R | 1R05 |
| V No basic domain | No | Classes I, II, V, and VI | ID1, ID4 | R | 6MGN |
| VI Proline in the basic domain | Yes | Classes I, II, V, and VI | HES1, HEY1 | R | 2MH3 |
| VII PAS domain | No | Class VII | ARNT, HIF1A | A or R | 5SY7 |

* Few members can homodimerize, including SRBEF1 and MAX [37]. Dimeric interactions for classes in gray are not summarized in this review. A: transactivator; R: transcriptional repressor. * Example of a Protein Data Bank (PDB) ID [38]. Class II homodimers and heterodimers are summarized in detail in this work. Refs. [6,21,22,37,39–41].

Early on, the bHLH TFs were also classified as groups A to D [42] based on phylogenetic sequence comparison of the bHLH motif and the DNA-binding specificity. Each of the four groups recognized a specific E-box sequence in the DNA. Group A included E-proteins and the TFs of Class II, defined above. Group B was composed of Murre’s TFs Classes III, IV, and VI and could be further subdivided due to an LZ motif’s presence or absence. PAS-domain proteins belonged to the C group, and ID proteins went into the D group.

Ledent and collaborators [43] expanded the Atchley and Fitch [42] classification above with two groups: The E group, now containing the Murre’s Class VI proteins, and the F group, which had an additional COE (Collier/Olf1/EBF) domain [43–45]. Moreover, numerous bHLH motifs from other organisms such as C. elegans and mouse were included in the phylogenetic analyses. These additions resulted in further classification of the bHLH TFs into orthology families [44], where the Atchley’s D group became members of the A group. Some classifications kept D TFs as an independent group [46].

Afterward, the complete amino acid sequences of the bHLH TFs of seven different species (human, mouse, rat, worm, fly, yeast, and plant) were used to carry out phylogenetic analyses that identified six new clades [47]. Clades 1 to 5 contained bHLH genes formerly classified as Classes I and II [22]. Clade 1 was made up primarily of mammalian genes previously considered to belong to Class II. Clade 2 included previous Classes I, II, and V. Whereas Clade 3 contained myogenic factors and some previous group II proteins.
Clade 4 concentrated on proteins with an additional LZ region, and some Clade 5 members contained genes with PAS domains. Clade 6 was specific to plant genes [47]. This analysis developed a phylogenetically precise relationship among bHLH genes and a new nomenclature based on the clade distribution [48].

Table 2 categorizes each factor according to the three bHLH TF classifications detailed above. As this review focuses only on vertebrates, a list of source model organisms for data summarized for each TF is included. Table 2 also lists each factor’s general transcriptional regulatory function, derived predominantly from transcriptional reporter assays. Caution should be taken when analyzing this information as unnoticed heterodimerization could be promoting context- or cell-dependent functionality [49–51].

Table 2. bHLH TFs’ classification and function.

| HGNC Gene Symbol and Aliases (a) | Classification | Function | Organism (b) |
|---------------------------------|----------------|----------|--------------|
| TCF3/E47 (E2-5)/ITF1           | Group A Class I | BHLH2/B  | E2A: A [52–54, 55]  |
| TCF3/E12                        | Group A Class I | BHLH2/B  | E2-5 [54], E47 [55], CR [55, 56, 59] |
| TCF4/E2-A/ITF2                  | Group A Class I | BHLH2/B  | A [52, 55, 56, 59, 61], CR [62, 63] |
| TCF4/E2-2B                      | Group A Class I | BHLH2/B  | CR [62, 63] |
| HEB/TCF12                      | Group A Class I | BHLH2/B  | A [52, 55, 56, 59] |
| MYOD1/MYOD/MYF3                 | Group A Class II | BHLH3/C  | A [65–66], TI [69] |
| MYOG/MYF4/Myogenin              | Group A Class II | BHLH3/C  | A [69, 70], TI [69] |
| MYF5                            | Group A Class II | BHLH3/C  | A [69] |
| TWIST1                          | Group A Class II | BHLH3/C  | A [69] |
| NEUROD1/BETA2/NEUROD            | Group A Class II | BHLH3/C  | A [97] |
| NEUROD2                         | Group A Class II | BHLH3/C  | A [97] |
| NEUROG1/NGN1/NEUROD3/Neurogenin | Group A Class II | BHLH3/C  | A [97] |
| NEUROG2/ATOH4/MATH4/Neurogenin2 | Group A Class II | BHLH3/C  | A [97] |
| NEUROG3/ATOH5/MATH5/Neurogenin3 | Group A Class II | BHLH3/C  | A [97] |
| ASCL1/MASH1                     | Group A Class II | BHLH3/C  | A [97] |
| ASCL2/MASH2                     | Group A Class II | BHLH3/C  | A [97] |
| ASCL3/SGN3                     | Group A Class II | BHLH3/C  | A [97] |
| ASCL4/HASH4                    | Group A Class II | BHLH3/C  | A [97] |
| ASCL5                           | Group A Class II | BHLH3/C  | A [97] |
| TAL1/ASH1                      | Group A Class II | BHLH3/C  | A [97] |
| TAL2                            | Group A Class II | BHLH3/C  | A [97] |
| LYL1                            | Group A Class II | BHLH3/C  | A [97] |
| NHLH1/HEN1/NSCL                | Group A Class II | BHLH3/C  | A [97] |
| NHLH2/HEN2/NSCL                | Group A Class II | BHLH3/C  | A [97] |
| MSC/Musclin/ABF-1/MyoR          | Group A Class II | BHLH3/C  | A [97] |
| TCF21/Capsulin/POD1             | Group A Class II | BHLH3/C  | A [97] |
| TCF23/OUT                      | Group A Class II | BHLH3/C  | A [97] |
| TCF24/OUT                      | Group A Class II | BHLH3/C  | A [97] |
| BHLH1A/BHLH2/BHLH1B/BHLH1C      | Group A Class II | BHLH3/C  | A [97] |
| BHLH22/BHLH23/BHLH24/BETA1      | Group A Class II | BHLH3/C  | A [97] |
| OLG1                           | Group A Class II | BHLH3/C  | A [97] |
| OLG2                           | Group A Class II | BHLH3/C  | A [97] |
| OLG3                           | Group A Class II | BHLH3/C  | A [97] |
### Table 2. Cont.

| HGNC Gene Symbol and Aliases (a) | Classification | Function | Organism (b) |
|---------------------------------|----------------|----------|--------------|
| **Class V** |           |    |            |
| BHLHE40/SHARP2/STRA13/DEC1 | Group A | Class II | BHLH5/E | R [152,153] | Hs, Mm, Rn |
| BHLHE41/SHARP1/DEC2 | Group A | Class II | BHLH5/E | R [154,155] | Hs, Mm, Rn |
| **ID1** | Group D | Class V | BHLH2/B | R [20,156] | Mm, Hs, Rn |
| **ID2** | Group D | Class V | BHLH2/B | R [157] | Mm, Hs |
| **ID3** | Group D | Class V | BHLH2/B | R [158] | Mm |
| **ID4** | Group D | Class V | BHLH2/B | R [159] | Mm |
| **Class VI** |           |    |            |
| HEY1/HRT1/CHF2/HERP2/Hers1 | Group E | Class VI | BHLH2/B | R [160,161] | Hs, Mm |
| HEY2/HRT2/CHF1 gridlock/HERP1 | Group E | Class VI | BHLH2/B | R [162] | Hs, Mm, Gg |
| HEYL/HERP3/HRT3 | Group E | Class VI | BHLH2/B | R [163,164] | Hs, Mm |
| HES1/HRT2/Xhairy1 | Group E | Class VI | BHLH2/B | A [165]. R | Hs, Mm, Rn, M |
| HES2 | Group E | Class VI | BHLH2/B | R [166] | Mm, Hs |
| HES3 | Group E | Class VI | BHLH2/B | R [169] | Xl, Hs (d) |
| HES4/Xhairy2 | Group E | Class VI | BHLH2/B | R [170] | Rn, Mm, Xl, Gg |
| HES5/ESR9 | Group E | Class VI | BHLH2/B | R [171] | Inhibits |
| HES6 | Group E | Class VI | BHLH2/B | R [172] | Hs, Mm, Xl |
| HES7 | Group E | Class VI | BHLH2/B | R [173] | Hs, Mm |
| HELT/MGN/HESL/MEGANE | Group E | Class VI | BHLH2/B | R [174] | Hs, Mm, Rn |

**Color key:**
- Binds DNA as homodimer
- Titrates E-proteins
- Titrates E-proteins and binds DNA as homodimer
- Transactivator and repressor
  - Transactivator (A)
  - Repressor (R)/Context dependent repressor (CR)
  - Transcriptionally inactive (TI)

(a) The HUGO Gene Nomenclature Committee (HGNC) approved gene symbol is followed by common synonyms and aliases, including names for other vertebrates. (b) Gg (Gallus gallus), Hs (Homo sapiens), Mm (Mus musculus), Rn (Rattus norvegicus), Xl (Xenopus laevis), M (monkey). (c) Sequesters TFs other than E-proteins. (d) No active Mm expression.

### 4. Dynamic Nature of the bHLH TFs

The bHLH TFs function cooperatively as homodimers (E47/E47), heterodimers (MYOD1/E47), trimers (TAL1/E47/LIM), or multimeric structures [107,124,127,177,178]. These protein–protein interactions within the bHLH family are highly dynamic and cell and context dependent [51]. This cooperativity impacts the function, DNA-binding preferences, cofactor interactions, subcellular localization, and interactions with other proteins [179,180]. Thus, the developmental fate of each cell and tissue is related to the composition of the functional bHLH dimers or multimers present [5,66,181].

Dimeric interactions within the bHLH TF family are summarized in Table 3 (parts A and B), Table 4 (parts A and B), and Table 5, specifically among bHLH protein classes capable of interacting with E-proteins. These TFs belong to Classes I, II, V, and VI as grouped by the Murre Lab. TFs in groups III and IV were not included as their transcriptional network has been recently reviewed [37]. The bHLH TFs also participate in interactions with TFs from other families, including, for example, the LIM-domain protein family, excellently reviewed elsewhere [182].

| Part A. | Heterodimers with E47 or E12 |
|---------|---------------------------|
| Class II TFs/Eprot | E47 | E12 |
| **MYOD1** | E2A: MS, Er(4), Ek(2), Ei(4), Ee(2), C(2), cIP(2), FS, qY2H, Sd, GST, Y2H, NI | Ei (5), Er, Ek, Ee, C(3), MIF, cIP(2), qY2H, Y2H, MS, NL, ChIP |
| **MYOG** | Er, Ek, Ei, cIP, qY2H [26,69,183] | Ei(3), Er, ChIP MIF, cIP, Y2H, MS [26,64,73,134,135,184,185] |
| **MYF5** | Ei, cIP, ChIP, qY2H [26,183,185] | Ei(2), cIP, MIF, ChIP, qY2H, MS [26,72,135,183,185] |
| **MYF6** | Er, Ei, Ei, cIP, qY2H [26,69,183] | Ei, MIF, cIP, ChIP, qY2H [26,183,185] |
| **MESPI** | Y2H [186] | |
| **MESP2** | Y2H [186] | |
| **FIGLA** | E2A: Ee(2) [74,75], Y2H [187] | Ee [74] |
| Part A | Heterodimers with E47 or E12 |
|-------|------------------------------|
| SCX   | clIP(2), Ee(2), Y2H(2), ChIP, Sd | Er, Ei(2), Y2H(2), MS [76,135,184,198] |
| TCF15 | TiP, Ei(2), clP [192], Y2H, MS, GST | Er, Ei(2) clP [84,182] |
| TWIST1| E2A: clP [192], Ei(2), GST, M2H | GST, clIP(3), clP, Ei, Sd [80,81,195–197] |
| TWIST2| GST, Sd, Ei(2), Y2H, clP | Y2H, clP, Ei, clP [191,197] |
| FERD3L| TCF3: clP, MS | GST, clP, Ei, M2H [91,197] |
| HAND1 | Y2H, GST, Ei(2), clP | GST, Y2H, M2H, Ei(2), Cl [91,197] |
| HAND2 | Y2H, GST, Ei(2), clP | GST, Y2H, M2H, Ei(2), Cl [91,197] |

| Part B | Heterodimers with TCF4 or TCF12 |
|-------|------------------------------|
| TCF4  | clP, Ei, Y2H | TCF4, SEC, GST [177,183] |
| TCF12 | E2A: clP(2), Ee, MS, GST, M2H, Cl | GST, clP, Ei(2) [183] |
| MYOD1 | Y2H, Ei(2) | Y2H, Ei(2) [183] |
| MYOG  | Y2H, Ei(2) | Y2H, Ei(2) [183] |
| FIGLA | clP, clIP(2) | Y2H, Ei(2) [183] |
| SCX   | Y2H, Ei(2) | Y2H, Ei(2) [183] |
| TCF15 | Ei(2), clP, M2H | Ei(2), clP, M2H [183] |
| TWIST1| E2A: clP(2), Ee, Y2H, GST | E2A: clP(2), Ee, Y2H, GST [183] |
| TWIST2| GST, Y2H, clP | GST, Y2H, clP [183] |
| FERD3L| Y2H, GST | Y2H, GST [183] |
| HAND1 | Ei(3), clP, Ei(2), GST [206] | Ei(3), clP, Ei(2), GST [206] |

**Table 3. Cont.**
Table 3. Cont.

| Part B | Heterodimers with TCF4 or TCF12 |
|--------|---------------------------------|
| HAND2  | Y2H(2), GST, Ee, Ei, M2H [30,94,157] | Y2H, GST, Ee(nDB) [30] |
| PTFA1  |                                   | Ei (2) [30,94,157] |
| NEUROD1| Ei (2) [29]                      |                           |
| NEUROD2| Ei(2), Ni, cIP [25,224]          | cIP, Ei [28]             |
| NEUROG1| cIP, Y2H, Ee [28]               | MS [210]                 |
| NEUROD4| (a)                             | (a)                      |
| NEUROD6| ASC1                              |                           |
| ATOH1  | ASC2                              |                           |
| NEUROG2| ASC3                              |                           |
| ATOH7  | ASC4                              |                           |
| NEUROG3| ASC5                              |                           |
| ATOH8  | BHLHA15                           |                           |
|       | NEUROD1                          |                           |
|       | NEUROD2                          |                           |
|       | NEUROG1                          |                           |
|       | NEUROG2                          |                           |
|       | NEUROG3                          |                           |
|       | NEUROG4                          |                           |
|       | TAL1                             |                           |
|       | TAL2                             |                           |
|       | LYL1                             |                           |
|       | NHLH1                            |                           |
|       | NHLH2                            |                           |
|       | MSC                              |                           |
|       | TCF21                            |                           |
|       | TCF22                            |                           |
|       | TCF24                            |                           |
|       | BHLHA19                          |                           |
|       | BHLHE22                          |                           |
|       | BHLHE23                          |                           |
|       | BHLHE40                          |                           |
|       | BHLHE41                          |                           |
|       | Color key:                       |                           |
|       | Both in vitro and in vivo        |                           |
|       | Only in vitro assays             |                           |
|       | Only EMSA                        |                           |
|       | Only in vivo assays              |                           |

* Due to numerous references for MYOD and TAL1 interactions, references are indicated here: MYOD-E47: E2A: MS [135]. Er(4), Ek(2), Ei(4), Ee(2), C(2), cIP(2), FS, Y2H, Sd, GST, Y2H, Ni [7,26,66–69,183,188,227–232]. MYOD-E12: Ei (5), Er, Ee, C(3), MiF, cIP(2), qY2H, Y2H, MS, Ni, ChIP [7,26,67,135,183–185,195,227,228,230,232,233]. TAL1-E47: E2A: Ei, GST, Y2H, cIP, MS [124,216,234]; Ee, Ek(2), C, cIP, ChIP, E2A: Y2H [120,127,181,225,234,235]. Numbers in parenthesis indicate independent experiments. Techniques (defined in Appendix A): Er = EMSA with recombinant protein. Ei = EMSA with in vitro translated protein. Ee = EMSA cell/nuclear extracts and super-shift or transfected cells. Ek = EMSA dissociation kinetics. cE = competitive EMSA. cIP: co-immunoprecipitation. GST = GST pulldown and related techniques. C = CASTing. Sd = sandwich assay. IF = colocalization by immunofluorescence. Cr = crystallography. Fw = far Western blot. CD = circular dichroism. F = FRET. FP = DNase I footprinting. w: weak interaction. nDB: no DNA binding. (a) No direct interaction was tested, but it is assumed from transactivation assays with a reporter gene [107]. (b) No direct proof of interaction suggested specific squelching mechanisms through reporter gene repression assay [145]. * The bHLH motif (66 amino acids) only has a one aa difference with HEN1, suggesting that it is also likely to heterodimerize with E2A [218].
Table 4. Heterodimeric interactions of Class II bHLH TFs with Classes II, V and VI TFs. Parts A and B.

| Part A. Class II-Class II Interactions | MYOD1 | MYOG | MYF5 | MYF6 | TWIST1 | HAND1 | HAND2 | NEUROD1 | NEUROG2 | NEUROG3 | ASCL1 | TAL1 | OLIG1 | BHLHE40 |
|---------------------------------------|------|-----|------|------|--------|-------|-------|---------|---------|---------|-------|------|------|--------|
| MYOD1                                 |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| MYOG                                  |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| MYF5                                  |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| MYF6                                  |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| MESPI                                 |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| MESP2                                 |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| FIGLA                                 |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| SCX                                   |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| TCF15                                 |      |     |      |      |        |       |       |         |         |         |       |      |      |        |
| TWIST1                                | dIP, GST [81] | GST [81] | GST [81] | GST [81] |        |       |       |         |         |         |       |      |      |        |
| TWIST2                                |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| FERD3L                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| HAND1                                 | cIP, F [193] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| HAND2                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| PTF1A                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROD1                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROD2                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROG1                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROD4                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROG2                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| NEUROG3                               |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| ATOH1                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| ATOH7                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| ATOH8                                 |        |     |      |      |        |       |       |         |         |         |       |      |      |        |
| BHLHA15                               | Ei (nDB), GST [115] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| ASCL1                                 | GST [112] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| ASCL2                                 | GST [112] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| ASCL3                                 | GST [112] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| ASCL4                                 | GST [112] |       |       |       |        |       |       |         |         |         |       |      |      |        |
| ASCL5                                 | GST [112] |       |       |       |        |       |       |         |         |         |       |      |      |        |
### Table 4. Cont.

#### Part A. Class II-Class II Interactions

| Class II Proteins | MYOD1 | MYOG | MYF5 | MYF6 | TWIST1 | HAND1 | HAND2 | NEUROD1 | NEUROG2 | NEUROG3 | ASCL1 | TAL1 | OLIG1 | BHLHE40 |
|-------------------|-------|------|------|------|--------|-------|-------|----------|----------|----------|-------|------|-------|---------|
| TAL1              |       |      |      |      |        |       |       |          |          |          |       |      | ****  |         |
| TAL2              |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| LYL1              |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| NHLH1             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| NHLH2             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| MSC               |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| TCF21             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| TCF23             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| TCF24             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| BHLHA9            |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| BHLHE22           |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| BHLHE23           |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| OLIG1             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| OLIG2             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| OLIG3             |       |      |      |      |        |       |       |          |          |          |       |      |       |         |
| BHLHE40           | cIP [155] |    |      |      |        |       |       |          |          |          |       |      |       |         |
| BHLHE41           | cIP(2), cE, GST(2) |  |      |      |        |       |       |          |          |          |       |      |       |         |

#### Part B. Class II-Class V or VI Interactions

| Class II Proteins | ID1 | ID2 | ID3 | ID4 | HEY1 | HEY2 | HEYL | HES1 | HES2 | HES4 | HES5 | HELT |
|-------------------|-----|-----|-----|-----|------|------|------|------|------|------|------|------|
| MYOD1             | ***** | qY2H, cIP, M2H [183] | cIP, M2H, cEMSA [158] | cIP, cE [160] | w: SSPC [194] |       |
| MYOG              | qY2H, M2H [182] | qY2H, M2H [182] | M2H [187] |      |      |      |      |      |      |      |      |      |
| MYF5              | cIP[183] | cIP[183] | cIP[183] |      |      |      |      |      |      |      |      |      |
| MESP1             |      |      |      |      |      |      |      |      |      |      |      |      |
| MESP2             |      |      |      |      |      |      |      |      |      |      |      |      |
| FIGLA             |      |      |      |      |      |      |      |      |      |      |      |      |
### Table 4. Cont.

#### Part B. Class II-Class V or VI interactions

| ID1 | ID2 | ID3 | ID4 | HEY1 | HEY2 | HEYL | HES1 | HES2 | HES4 | HES5 | HELT |
|-----|-----|-----|-----|------|------|------|------|------|------|------|------|
| SCX |     |     |     |      |      |      |      |      |      |      |      |
| TCF15 | cIP [192] |     |     |      |      |      |      |      |      |      |      |
| TWIST1 |     |     |     |      |      |      |      |      |      |      |      |
| TWIST2 |     |     |     |      |      |      |      |      |      |      |      |
| FERD3L |     |     |     |      |      |      |      |      |      |      |      |
| HAND1 |     |     |     |      |      |      |      |      |      |      |      |
| HAND2 |     |     |     |      |      |      |      |      |      |      |      |
| PTF1A |     | MDS [200] |     |      |      |      |      |      |      |      |      |
| NEUROD1 |     |     |     | GST [90] | GST [90] | GST [90] | GST [90] | GST [90] | GST [90] | GST [90] | GST [90] |
| NEUROD2 |     |     |     |      |      |      |      |      |      |      |      |
| NEUROG1 |     |     |     | cIP [240] | cIP [240] | cIP, Y2H, GST [240] | cIP [240] | cIP [240] | cIP [240] | cIP [240] | cIP [240] |
| NEUROD4 |     |     |     | w: cIP [241] | cIP [241] | cIP [241] | cIP [241] | cIP [241] | cIP [241] | cIP [241] | cIP [241] |
| NEUROD6 |     |     |     |      |      |      |      |      |      |      |      |
| ATOH1 |     |     |     |      |      |      |      |      |      |      |      |
| NEUROG2 |     |     |     |      |      |      |      |      |      |      |      |
| NEUROG3 |     |     |     |      |      |      |      |      |      |      |      |
| ATOH7 |     |     |     |      |      |      |      |      |      |      |      |
| ATOH8 |     |     |     |      |      |      |      |      |      |      |      |
| BHLHA15 |     |     |     |      |      |      |      |      |      |      |      |
| ASCL1 |     |     |     |      |      |      |      |      |      |      |      |
| ASCL2 |     |     |     |      |      |      |      |      |      |      |      |
| ASCL3 |     |     |     |      |      |      |      |      |      |      |      |
| ASCL4 |     |     |     |      |      |      |      |      |      |      |      |
| ASCL5 |     |     |     |      |      |      |      |      |      |      |      |
| TAL1 |     |     |     |      |      |      |      |      |      |      |      |
| TAL2 |     |     |     |      |      |      |      |      |      |      |      |
| LYL1 |     |     |     |      |      |      |      |      |      |      |      |
| NHLH1 |     |     |     |      |      |      |      |      |      |      |      |
### Part B. Class II-Class V or VI interactions

| ID1  | ID2  | ID3  | ID4  | HEY1 | HEY2 | HEYL | HES1 | HES2 | HES4 | HES5 | HELT |
|------|------|------|------|------|------|------|------|------|------|------|------|
| NHLH2|      |      |      |      |      |      |      |      |      |      |      |
| MSC  | TCF21| TCF23| TCF24| BHLHA9| BHLHE22| BHLHE23| OLIG1| OLIG2| OLIG3| BHLHE40| BHLHE41| cIP, b2H, IF [146] | + | ++ |
|      |      |      |      |      |      |      |      |      |      |      |      | GST, cIP [132] |

Color keys and abbreviations are as in Table 3. The pale-yellow area does not show interactions to avoid repeated data from the white area. Interactions with numerous references are detailed here: BHLHE41-MYOD1: cIP(2), cE, GST(2) [154,155,221]. HAND2-HAND1: Y2H, GST, F, M2H [90,193]. ASCL1-NEUROG2: Y2H, cIP, GST, Ei(2), Ee [107,206,244]; for [206], no DNA binding was observed with EMSA with in vitro translated protein. OLIG2-NEUROG2: cIP, M2H, Y2H, GST [148,150]. LYL1-TAL1: MS, cIP, ChIP, ChIP-seq [124,127,245]. MYOD1-ID1: cIP(3), Y2H, GST, M2H(2), qY2H [157,176,183,244,246]. OLIG1-ID4: BMFCS [247], cIP, b2H, IF [146]. OLIG2-ID4: cIP, b2H, IF [146]. ASCL1-HELT: Y2H, cIP, IF [244].
Table 5. Homodimeric interactions of Class I and II bHLH TFs.

| Factor | Homodimer | EMSA | 2H | GST | cIP | MS | Biophysical | Other | Function (a) |
|--------|-----------|------|----|-----|-----|----|-------------|-------|--------------|
|        |           |      |    |     |     |    |             |       |              |
| Class I|           |      |    |     |     |    |             |       |              |
| TCF3/E47 | Y         | Y: Ei [7,8], Er [227], Eo [227] | Y: [194] | Y: Cr [248], FS, CD [231] | Y: C, Ek [227]; MIF [8] | A [52–57] |
| TCF3/E12 | Y         | Y: Y2H [184] | Y: [135] | Y: CD [177] | Y: C, Ek [227] (b) | A [52,57,60] |
| TCF4 | Y         | Y: Ei [249], Eo [224] | Y: [222] | Y: Cr, (f) [249] |             | A [52,56,57,60,61] |
| TCF12 | Y         | Y: Ei [64] |             |             |             |             |
| Class II|           |      |    |     |     |    |             |       |              |
| MYOD1 (e) | Y         | ** | w: qY2H [183] | Y: [135] | Y: Cr [250], CD, FS [231] | Y: MIF [70] | TI [69] |
| MYOG (e) | Y         | Y: Er [72], wq: Er [69] | w: qY2H [183] |             |             |             |
| MYF5 (e) | Y         | Y: Er [72] | w: qY2H [183] | N: qY2H [183] | N: Y2H [186] | N: Y2H [186] | TI [69] |
| MYF6 (e) | Y         | N: Y2H |             |             |             |             |          |
| MESP1 | ?         | N: Ei, Er [76], Y: Ei [251] |             |             |             |             | A [251] |
| MESP2 | ?         |             |             |             |             |             |              |
| FIGLA | ?         |             |             |             |             |             |              |
| SCX | ?         | N: Ei, Er [76], Y: Ei [251] | N: Y2H [186] |             |             |             |              |
| TCF15 | ?         | N: Ei (3) [29,190,191] |             |             |             |             |              |
| TWIST1 | Y         | Y: Ei (2) [196,197] | Y: [81] | Y: [236] | Y: [236] | Y: FRET [193], (g) |              |
| TWIST2 | ?         | N: Ei [184] |             |             |             |             |              |
| FERD3L | ?         | N: Ei [84] |             |             |             |             |              |
| HAND1 | Y         | N: Ei [36], Y: M2H, Y2H [90] | Y: [90] | Y: E-box: CD |             | Y: M2H, C(nDB) [90] | A [78,199] |
| HAND2 | Y         | N: Ei [31], Eo [36] | Y: Y2H w: M2H [91] | Y: [90,91] | Y: [92] | N: C [91] | TI [91] |
| PTF1A | ?         | N: Ei [94,201], Eo [93] |             |             |             |             |              |
| NEUROD1 | ?         |             |             |             |             |             |              |
| NEUROD2 | ?         |             |             |             |             |             |              |
| NEUROG1 | ?         |             |             |             |             |             | Y fuzzy E-box: CD [253] |
| Factor   | Homodimer | EMSA | 2H | GST | cIP | MS | Biophysical | Other | Function (a) |
|----------|-----------|------|----|-----|-----|----|-------------|-------|--------------|
| NEUROD4 | ?         | N: Ei [208] |    |     |     |    |             |       | A? [103]     |
| NEUROD6 | Y         | Y: Er [105], Ee [104] |    |     |     |    |             |       | A [105]      |
| ATOH1   | Y         | N: Er [106], Y: Ee [28] |    |     |     |    |             | Y: 210 |              |
| NEUROG2 | Y         | N: Ei [206] | N: Y2H [150] | Y: [31] | Y: ChIP [31](c) | N: CD (nDB) [253] |             |              |
| NEUROG3 | N?        | N: Ei [108] |    |     |     |    |             |       |              |
| ATOH7   | ?         | Y: Er [111] |    |     |     |    |             |       |              |
| ATOH8   | ?         |        |    |     |     |    |             |       |              |
| BHLHA15 | Y         | Y: Ei [115, 116,213]; Ee [254] | Y: [116] | Y: [115, 254] | Y: C [115], BMFCS [254] | A [115,254], BMFCS [254] | A [107], R [111] |              |
| ASCL1   | Y         | Y: Ei [107] | Y: Y2H [244] | Y: [244] | Y: CD [255] | Y: Ek [214] | A [111] |              |
| ASCL2   | ?         | N: Ei [39] | N: Ei [119] | Y: Y2H [119] | Y: [119] | N: C [119] | R [119] |              |
| ASCL3   | Y         |        |    |     |     |    |             |       |              |
| ASCL4   | ?         |        |    |     |     |    |             |       |              |
| ASCL5   | ?         |        |    |     |     |    |             |       |              |
| TAL1    | Y         | N: Ei [216], Er [177] | N: qY2H [183] | N: [125,127] | Y: [124] | Y: CD [177] |       |              |
| TAL2    | ?         | N: [217] | N: qY2H [183] | N: qY2H [183] | Y: [127] | Y: [127] |       |              |
| LYL1    | Y         |        |    |     |     |    |             |       |              |
| NHLH1   | Y         | Y: Ei, Ee [130,218] | Y: M2H [130] | Y: [218] | Y: C [218] | A [130] |        |              |
| NHLH2   | Y         | Y: Ee [131] | Y: Ei [133,134] | Y: [131] |       | A [131] |        |              |
| MSC     | Y         |        |    |     |     |    |             |       | R [133,134] |
| TCF21   | N?        | N: Ei [219] | N: Y2H [137,220] |     |     |     |       |              |
| TCF23   | ?         |        |    |     |     |    |             |       |              |
| TCF24   | ?         |        |    |     |     |    |             |       |              |
| BHLHA9  | ?         | Y: Y2H [141] |     |     |     |    |             |       |              |
| BHLHE22 | Y         | N: [143] | Y: [142] | Y: ChIP [142](d) | R [142,144] | R [145] | R [147] |              |
| BHLHE23 (c) | Y | Y: Ei [147] | Y: Y2H [150], M2H [148] | Y: [150] | Y: [148] | Y: FCCS [256] | R [149,150,257] |              |
| OLIG1   | Y         | Y: Ei [150] |     |     |     |    |             |       |              |
| OLIG2   | Y         | Y: Ei [150] | Y: Y2H [150], M2H [148] | Y: [150] | Y: [148] | Y: FCCS [256] | R [149,150,257] |              |
| OLIG3   | ?         |        |    |     |     |    |             |       |              |
| BHLHE40 | Y         | Y: Ei [153, 155,237] | Y: [153] |       |       |     |             |       | R [153,155] |
### 5. The Current Functional bHLH Model

The Murre classification scheme (Table 1) was selected for this review because it separates E-proteins from tissue-specific TFs and classifies ID proteins and the HES family in independent groups. Publications by the Murre Lab proposed a general way in which bHLH TFs function and interact. This model is widely accepted by previous and current publications in the field [14,25,62,129,138]. Briefly, Class I proteins were usually transactivators as homodimers or heterodimers with Class II, tissue-specific proteins. Class V proteins repressed many Classes I and II proteins, primarily by sequestering E-proteins, and Class VI proteins were transcriptional repressors [22].

Predictions of the functionality of bHLH TFs could be made based on the classification above and the other phylogenetic classifications; however, this could be misleading as each bHLH dimer’s function depends on the protein–protein interactions established. Thus, the relevance of this review originates from the need to summarize experimentally corroborated dimeric interactions among this TF family.

From Table 2, the following can be concluded: E-proteins are indeed transactivators as homodimers. However, E47 and E2-2 have also been reported to be context-dependent repressors. On the other hand, of 48 Class II TFs analyzed, 10 are only reported as transactivators, 12 only as repressors, 21 as both transactivators and repressors (or transcriptionally inactive dimers), and 5 remain untested. Furthermore, the majority of the Class II TFs can

### Table 5. Cont.

| Factor | Homodimer | EMSA DNA binding | 2H | GST | cIP | MS | Biophysical | Other | Function (a) |
|--------|-----------|------------------|----|-----|-----|----|-------------|-------|-------------|
| BHLHE41 | Y         | Y: Ei [155,221] | Y: [155] | (Independent experiments) | Transactivator (A) | |
| Color key: | only EMSA | No DNA binding | DNA binding | Opposite DNA binding results | Repressor (R) | A and R | |
| only in vitro assays | | | | | | | |
| only in vivo assays | | | | | | | |
| In vitro and in vivo | | | | | | | |
| Untested | |

* N: Ei [7] (N), [230] (N), [184] (Y); Er [227] (N); w: Er [67]. ** w: Ei [230], Er (4) [65,227,231]. q: Er [69]. N: Ei [7]. (a) Consider the possibility of confounding results due to heterodimerization with endogenous proteins in reporter transfection assays. (b) Chromatographic properties on gel filtration suggest a stable homodimer with no DNA binding by EMSA [227]. (c) There was 95% bHLH sequence identity with bHLHBS [145]. (d) For BHLHE22 and NGN2, DNA binding was not observed with EMSA but was demonstrated with ChIP. The requirement for additional factors for DNA binding cannot be eliminated. All myogenic factors had weak interactions with duplex DNA but bound quadruplex DNA well. (f) Fluorescence polarization and isothermal titration calorimetry. (g) Non-reducing SDS-PAGE of co-transfected protein extracts. (h) Protein–protein and DNA–protein interaction ELISAs. (i) Low phosphorylation. (j) The factor was tested as a homodimeric transactivator with hard-to-dissect reporter assays due to possible dimerization with endogenous proteins. Techniques: Er = EMSA with recombinant protein. Ei = EMSA with in vitro translated protein. Ee = EMSA cell/nuclear extracts and super-shift or transfected cells. Ek = EMSA dissociation kinetics. cE = competitive EMSA. cIP: coimmunoprecipitation. GST = GST pulldown and related techniques. C = CASTing. Sd= sandwich assay. IF = colocalization by immunofluorescence. Cr = crystallography. Fw = far Western blot. CD = circular dichroism. F = FRET. FP = DNase I footprinting. MS = mass spectrometry. BMFCS = bi-molecular fluorescence complementation system. FCCS = fluorescence cross-correlation spectroscopy. w: weak interaction. nDB: no DNA binding. q: quadruplex DNA.

In many cases, phosphorylation of the bHLH TF regulates its dimerization. NEUROG2 homodimers are efficient transactivators. However, when NEUROG2 is phosphorylated, it heterodimerizes with E47, reducing its transactivator capacity [31]. For OLIG2, its phosphorylation promotes homodimerization and transcriptional repression. OLIG2 dephosphorylation promotes heterodimerization with NEUROG2, a relevant process required for the motor neuron-oligodendrocyte fate switch [148]. Table S1 provides references for bHLH factors known to be regulated by phosphorylation.
dimerize with E-proteins (Table 3, parts A and B). Nevertheless, this interaction with E-proteins does not always result in transactivation, as 19 class II TFs can sequester E-proteins in transcriptionally inactive dimers, and factors such as TCF21 and NEUROG3 can repress transcription as DNA-binding heterodimers with Class I proteins (Table 2). Likewise, some tissue-specific TFs can heterodimerize with bHLH TFs other than E-proteins (Table 4) or form homodimers (Table 5) with positive and negative transcriptional effects (see below).

6. bHLH Dimeric Interactions: The Importance of the Experimental Approach

Diverse experimental approaches, including in vivo and in vitro assays, have defined the dimeric interactions of bHLH transcription factors. Appendix A briefly describes these assays, classifying them as biochemical, biophysical, or genetic.

The most common in vitro assay for testing dimeric bHLH interactions in the presence of DNA is the electrophoretic mobility shift assay (EMSA). This approach has been used since the discovery of the bHLH TFs and has demonstrated most E-protein homodimeric and heterodimeric interactions with Class II bHLH TFs. These interactions include all DNA-binding myogenic and neurogenic bHLH heterodimers. A major drawback of EMSA is that it cannot detect DNA-independent interactions or dimers that bind non-consensus or untested DNA sequences. Thus, unless a broader repertoire of DNA sequences was tested, such as in the CASTing assay [258], the possibility of interaction with another sequence (e.g., ESE-box) cannot be eliminated. Furthermore, a negative result in the EMSA only indicates that the dimer may not be binding to the DNA, as was the case for ASCL3 homodimers [119].

Some bHLH TFs dimers were discovered by alternate in vitro approaches, including GST-pulldown (GST), methylation interference footprinting (MIF), co-immunoprecipitation (coIP, also considered an ex vivo assay, Appendix A), X-ray mass spectrometry (MS), and circular dichroism (CD). The most common in vivo approach is the yeast two-hybrid (Y2H) assay, which has defined multiple E-protein dimeric interactions. Other in vivo approaches include the mammalian two-hybrid (M2H), the site-specific photocrosslinking (SSPC), and the fluorescence resonance energy transfer (FRET) assays. Excellent reviews elsewhere state the advantages and drawbacks of diverse protein–protein interaction methodologies [259,260].

MYOD and HAND1 are members of the select group of TFs that have confirmed dimeric interactions through multiple independent techniques, including EMSAs, coIPs, X-ray MS, and CD (Tables 3–5). TFs whose dimeric interactions have been verified using in vivo and in vitro assays are color-coded in yellow in the tables.

The opposite situation is observed for MESP1, a TF whose dimeric interactions have only been analyzed with a single experimental technique, the Y2H. Even though most bHLH TFs have at least one verified interaction partner, a varied and complementary repertoire of experiments confirming dimeric interactions is unavailable for all TFs (Tables 3–5). This poor characterization of the TFs’ dimeric partners results in uncertainty about the biological significance of the interaction and is observed for other factors such as MESP2, FERD3L, NEUROG1, ASCL4, and OLG3. The color code in Tables 3–5 indicates purple for dimeric interactions that have only been analyzed with EMSA, green for interactions only tested with in vivo assays, blue for dimers tested only with in vitro approaches, and yellow for interactions tested with both, in vivo and in vitro assays.

Tables 3–5 and S2–S5 summarize the techniques used to define the bHLH TF homodimeric and heterodimeric interactions. When diverse experimental approaches are used, the interactions can be confirmed unequivocally. Positive or negative interaction results obtained with a particular technique may be influenced by the conditions tested: e.g., whether the proteins were purified, in vitro synthesized, expressed in a specific cell type, co-expressed with other factors, or tested in the presence of DNA or isolated environments. Furthermore, the strength and stability of the interaction tested can also affect the outcome of the experiments [259]. Balancing the available information on the experimen-
tal approaches reporting dimeric interactions will help the scientist assess the biological significance of the dimeric interaction of interest.

Additionally, when experimenting with in vitro translated proteins and recombinant bacteria-synthesized proteins, the protein–protein interactions may not be observed due to the requirement for specific posttranslational modifications or accessory proteins (e.g., LIM-domain proteins). For example, the interaction demonstrated by coIP between ATOH8 and NEUROD1 could not be reproduced using in vitro translated proteins [100]. Similarly, ATOH1 homodimers were confirmed with MS and cell extract EMSAs; however, EMSAs utilizing recombinant proteins did not find the interaction [28,106]. The specific reason for these experimental discrepancies remains to be studied.

In vivo assays preserve the native surrounding in which the interaction takes place. However, these assays also have drawbacks, such as the expression under non-physiological conditions (e.g., heterologous) and the influence of the cell context. Sometimes, an interaction can be observed in one cellular context but not in another. Reasons for these inconsistencies could be a requirement for additional interacting factors or an altered bHLH network composition due to a TF overexpression. TAL1 is an example of a TF capable of activating or repressing transcription in a context-dependent manner through differential interactions with HDACs and HATs [122–124] and sequestering E-proteins from other bHLH TFs such as MYOD1 [125].

In the transcriptional reporter assays, the most common approach to define the function of the dimeric bHLH TFs, the main drawback is the presence of a specific endogenous pool of bHLH TFs in the cell. This TF pool may contain TFs able to influence the function of the TF tested, a condition that must be considered by the scientist when analyzing homodimeric TFs.

7. Heterodimeric Interactions among bHLH TFs of Classes I, II, V and VI

In compiling this review, information was gathered about the dimeric interactions of bHLH TFs from individual publications since their discovery at the end of the 1980s. This exhaustive literature search was complemented by a manual search in global protein–protein interaction databases to guarantee a thorough summary of the bHLH dimer diversity. These web databases summarize experimentally corroborated and predicted vertebrate protein–protein interactions, with none of them being devoted to TFs or specifically to bHLH TFs. For this work, IntAct [261], String [262], the Bioplex Interactome [263], and the human interactome database [264] were queried. Only the experimentally confirmed interaction data were included.

Table 3 (parts A and B) shows heterodimeric interactions among all Class II TFs and the E-proteins TCF4, TCF12, and the two most prominent alternative splicing variants of TCF3: E12 and E47. It was found that 87% of the tissue-specific TFs can interact with either E12 or 47. Furthermore, 30% of the class II TFs can interact with all three E-proteins. It derives from here that E-proteins can replace each other’s functions. However, it is established that individual E-proteins are better partners than others for specific Class II TFs [25–35]. The E-protein–Class II TF interactions are the best characterized in the family and are generally considered to support transcription. However, Table 2 shows that multiple tissue-specific factors can sequester E-proteins and result in adverse transcriptional effects. Furthermore, gaps in Table 3 exemplify interactions that remain to be tested.

Class I TFs can also heterodimerize among each other, still functioning as transactivators (Table S2). Unfortunately, these heterodimers are poorly characterized, and there are no reports yet comparing the functionality of Class I homodimers with heterodimers.

Besides heterodimerizing with E-proteins, some bHLH TFs also form heterodimers with other Class II, V, and VI TFs (Table 4 parts A and B, and Table S3). This diversity of dimeric interactions alters the TFs functionality accordingly. For example, MYOD1 functions as a transactivator when heterodimerizing with E47 or E12 [50,66]. However, MYOD1 cannot transactivate as a homodimer [69] or when heterodimerizing with TWIST1 [81], bHLHE41 [154,221], HEY1 [101], and ID proteins [20,183]. ASCL1 homodimers and het-
erodimers with NEUROG2, HELT, or E12 function as transactivators [107,114,244]. However, experimental evidence exists for heterodimeric interactions between ASCL1 and HAND1 [90] or HES5 [170]. These heterodimers block the activity of ASCL1. From analyzing Table 4, 91% of the Class II–Class II TF heterodimers are transcriptionally inactive or repressive (Table S4). The only exceptions are the ASCL1/NEUROG2 dimer that transactivates Dll3 [107] and TAL1/LYL1 [127,245]. These two heterodimers transactivate through cooperation with additional factors [127,245]. The Class II factors TWIST1 [80,82], HAND1 [88–90], and TAL1 [125] can sequester other Class II factors. OLG2 is a Class II protein with a repressor domain that can repress other Class II factors [148,150], homodimerize, or heterodimerize with E-proteins [146,150]. In contrast, 100% of Class II TFs interactions with ID proteins and 95% of the interactions with Class VI TFs, negatively affect transcription (Tables 4 and S3). The Class V family, characterized by the absence of the basic DNA-binding domain, operates by sequestering E-proteins in non-DNA-binding heterodimeric complexes (Table S3). ID proteins can also establish non-functional dimers with Class II and VI TFs (Table S3). The Class VI family groups repressors [22,265], which structure homodimers and heterodimers with TFs from Classes II, V, and VI (Table S3). For instance, the best-characterized family member, HES1, heterodimerizes with multiple partners to block their activity or form dimeric repressors. These partners include E-proteins, MYOD1, PTF1, NHLH2, HEY1, HEY2, HEYL, and IDs (Table S3). Thus, whereas Class II factor interactions with E-proteins can generate transactivators or titrate Class I TFs, Class II TFs’ interactions with Class V, VI, and other Class II factors generally interfere with transcriptional activation.

The comprehensive 2019 review by Murre [6] summarized the detailed role of bHLH TFs in various pathways and clearly stated that the dynamics of the bHLH gene expression dictates the developmental choice. For many bHLH TFs, though, it did not emphasize the dimeric form involved. Likewise, studies only assessing the regulatory role of a Class II TF as a single entity, with no information about the dimerization partner, are common [251,266–273]. These omissions probably are because the dimeric partner involved is usually a ubiquitous TF such as the E-proteins, which are considered a platform for regulating a broad set of genes [5]. Furthermore, the tissue-specific regulators (Class II TFs) are usually responsible for fine-tuning gene expression, even when heterodimerizing with E-proteins. However, because the dimer composition is very variable (Tables 3–5), it is proposed that future publications in the field should state the composition of the dimeric (or multimeric) bHLH TFs involved. As an example, in a study of the role of the bHLH TF SCX in tissue fibrosis, it was concluded that the relevant bHLH dimer is SCX/E47, as SCX by itself did not have a role in the experiments tested [274].

8. bHLH TF Homodimers

Homodimerizing tissue-specific bHLH proteins in Drosophila were described as transcriptionally inactive [275,276]. These TFs became active upon heterodimerization with E-proteins. MYOD1 could also exist as a homodimer [39,66]. Experiments demonstrating MYOD1 homodimerization include X-ray crystallography [250], CD [231], Y2H [183], and LC-MS/MS [135]. The MYOD1 homodimers were transcriptionally inactive because their duplex DNA binding was compromised [8]. It was later established that MYOD1 and other myogenic bHLH TF homodimers preferred to bind quadruplex DNA instead of duplex DNA [69]. These DNA structures are now known to have a biological role, usually interfering with transcription [277]. Unfortunately, only the myogenic factors have been tested for binding this type of DNA structure [69]. Thus, further research in this area is required to determine whether G-quadruplex binding is a common way of inhibiting transcription by bHLH homodimers.

No systematic reviews about bHLH TF homodimerization exist. Table 5 summarizes the available homodimer information for 5 Class I and 48 vertebrate Class II bHLH TFs. From there, 21 Class II factors have experiments supporting homodimerization through consistent results using in vivo and in vitro approaches (yellow). Table 5 also indicates
whether the experimental evidence supports (Y) or does not support (N) homodimers or when the experimental evidence is inconclusive (question mark).

Homodimers of the myogenic factors (MYOD1, MYOG, MYF6) and six other factors (ASCL3, BHLHE22, MSC, OLIG2, BHLHE40, and BHLHE41) are transcriptionally inactive or act as repressors. Within this group, only ASCL3 does not bind duplex DNA as a homodimer. In contrast, five TFs bind DNA to remain transcriptionally inactive or repress gene expression (MSC, BHLHE22, OLIG2, BHLHE40, and BHLHE41). Thus, some Class II homodimers affect gene expression negatively through diverse mechanisms that can be dependent or independent of DNA binding, including the possible sequestration of components of active dimers.

BHLHA15 homodimers can transactivate or repress transcription through direct DNA binding in a context-dependent manner [115,116,254]. HAND1 and HAND2 homodimers cannot bind DNA. However, there is inconclusive evidence about their transcriptional roles [85,91,199].

Six Class II homodimers can function as transactivators through direct DNA binding: NEUROD6, NEUROG2, BHLHA15, ASCL1, NHLH1, and NHLH2. Thus, Class II homodimers can be both transcriptionally inactive and active. On the other hand, homodimerization of 27 (of 48) class II TFs is uncertain, either because it has never been tested or because the experimental evidence is inconclusive. The untested factors include FIGLA, NEUROD1, NEUROD2, ATOH8, ASCL4, ASCL5, TCF23, TCF24, BHLHE23, OLIG1, and OLIG3 (gray in Table 5). Similarly, homodimeric interactions for 16 Class II factors are inconclusive because interactions have only been tested utilizing EMSA (purple) or because independent experiments are insufficient.

Table S5 enlists homodimeric interactions for 4 Class V and 11 Class VI bHLH TFs. All Class V proteins are considered not to homodimerize. However, a splicing variant of ID1, ID1.25, was observed in adult cardiac myocytes and vascular smooth muscle cells [246]. ID1.25 preferentially forms homodimers and probably regulates the sequestering activity of ID1 [246].

Finally, 9 of 11 Class VI factors function as homodimers repressing transcription (Table S5). Homodimerization for HES7 has not been tested, and the evidence for HESL homodimers remains inconclusive. Class VI factors can repress by DNA binding or sequestration of other factors when structuring heterodimers [15,278].

9. Conclusions

Although bHLH TFs have been studied for over 30 years, there remain extensive gaps in our knowledge either because some dimeric interactions have never been tested or due to the inherent limitations of the techniques used. Furthermore, due to the bHLH TFs’ ability to interact with multiple partners, dissecting the function of each dimer pair requires carefully designed experiments. I anticipate that the field will be accelerated by increasingly powerful technologies such as cryo-electron microscopy/tomography and genome-wide interactomes in different cell types and conditions. Another layer of complexity is added by the fact that alternate dimerizing partners are usually co-expressed in vivo, establishing a dynamic pool of TFs, whose balance defines the outcome of the assays. This indicates the need for more live-cell approaches to allow the visualization of interaction dynamics and computational approaches using available dimer structures to predict bHLH interactions [279,280] and study the energy of the interaction landscape for bHLH homodimers and heterodimers [281,282]. Keeping this in mind, the available data presented in the tables support two major additions to the current functional bHLH TFs model (Figure 2). First, Class II factors’ interactions with bHLH TFs other than E-proteins usually result in adverse transcriptional effects. Second, homodimers of Class II TFs are common and have both positive and negative transcriptional effects. Positive effects are associated with DNA binding, whereas adverse effects can be independent of or dependent on DNA binding, including binding to G-quadruplex DNA structures. Because the study of bHLH TFs is such an active and productive field of investigation, the years ahead will likely
bring ever-increasing insight into sophisticated networks of gene regulation contributing to human development, health, and disease.

**Figure 2.** The diversity of dimeric interactions among different bHLH TF classes. Examples of dimeric interactions formed between bHLH TF members of different classes are shown. Examples of the proposed additions to the current model of bHLH TF dimers are highlighted in a blue area. Folded arrows in DNA indicate transactivating or repressing abilities. Interactions independent of DNA binding usually block transcription by factor sequestration.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/ijms22312855/s1.

**Funding:** This research was funded by Consejo Nacional de Ciencia y Tecnología (CONACYT) FONDO SECTORIAL DE INVESTIGACIÓN PARA LA EDUCACIÓN. Ciencia Básica. [CB2017-2018 A1-S-8737] to A.L.T-M.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** I thank Lorraine Pillus and Xue Bessie Su for their valuable comments and critical reading of this work. I also thank members of the Cell Biology Laboratory at INER for useful suggestions.

**Conflicts of Interest:** The author declares no conflict of interest. The funder had no role in the design of the study or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

**Appendix A. Methods for Detecting Protein–Protein Interactions of bHLH TFs**

**Appendix A.1. In Vitro Interactions**

Electrophoretic Mobility Shift Assay (EMSA, biochemical): Detects protein–nucleic acid interactions through slower electrophoretic mobility of the complex compared to the free nucleic acid [227,230].

The protein tested to interact with DNA can be derived from the following sources:

- Cell/nuclear extracts. Detection of a specific protein in the mixture can be achieved by incubation with antibodies that produce a super-shift or slower electrophoretic mobility in comparison to the protein–nucleic acid complex by itself.
- Recombinant purified proteins
- In vitro transcribed/translated proteins.

The DNA templates tested include the following:

- Single short DNA probes containing one or multiple protein-binding sequences (e.g., E-boxes, N-boxes, or ESE-boxes)
- Multiple random DNA-binding sequences (CASTing: Cyclic Amplification and Selection of Targets) [227,258].
Competitive EMSA (cEMSA, biochemical): Detects the disappearance of a shifted band when a protein–nucleic acid interaction is lost by incubation with another protein that can interact with a protein in the DNA–protein complex [143,158].

GST-Pulldown (biochemical): A protein fused to GST (bait) is immobilized to capture proteins that interact [283]. Interacting proteins can be recombinant, in vitro translated, or derived from cell lysates. Bait proteins can be immobilized utilizing alternative fused proteins.

Mass Spectrometry (MS, biophysical): Analytical chemistry method that measures the mass-to-charge ratio of molecules present in a sample. Proteins are identified from the mass spectra using computational methods [284].

X-ray Crystallography (biophysical): Determination of atomic-resolution structures by analyzing the diffraction of X-rays by crystals of a purified protein or complex [285].

Circular Dichroism (CD, biophysical): Spectroscopic method that characterizes protein structures by measuring differences in absorption of left and right circularly polarized light [286].

Footprinting (biochemical): Detects sequence-specific DNA binding when a purified protein or protein complex protects DNA from degradation by DNase I [287].

Methylation Interference Footprinting (MIF, biochemical): Identifies the exact DNA sequence for binding when a protein cannot bind its recognition sequence when DNA is methylated. Chemical cleavage distinguishes unprotected regions from protected sequences [73].

Enzyme-Linked Immunosorbent Assay (ELISA, biochemical): DNA–protein and protein–protein interactions can be detected by immobilizing DNA or a bait protein in microwell plates. Binding partners are detected from cell lysates through antibody interactions and enzymatic reactions [212,286].

Far Western Blot (biochemical): Proteins blotted to a membrane from an SDS-PAGE are probed for direct interaction with another protein that can be labeled or detected indirectly with specific antibodies [288].

Sandwich Screening Procedure (biochemical): Relies on protein–protein interactions to generate a specific DNA-binding activity. Recombinant proteins are immobilized in nitrocellulose and incubated with a “bait” protein. Incubation with labeled E-box probes confirmed the interaction between bHLH heterodimers and DNA [188].

Co-immunoprecipitation (co-IP, biochemical): Identifies protein–protein interactions when a bait-specific antibody is used to co-precipitate binding partners [289]. It is also considered an ex-vivo approach as the interaction occurs in vivo, whereas its detection occurs in vitro.

Appendix A.2. In Vivo Interactions

Yeast Two-Hybrid (Y2H, genetic): A reporter gene is activated when a bait protein fused to the DNA-binding domain of the transcription factor Gal4 is interacting with a prey protein fused to the Gal4 activation domain [290].

Bacterial Two-Hybrid (B2H, genetic): Adapted Y2H in bacteria. Avoids requirement for nuclear compartmentalization of proteins tested [291].

Mammalian Two-Hybrid (M2H, genetic): Adapted from Y2H in mammalian cells. Studies interactions in native context [291].

Fluorescence Resonance Energy Transfer (FRET, biochemical): When two proteins are interacting, energy is transferred from a bait protein fused to a donor fluorophore, upon excitation, to a prey protein fused to an acceptor fluorophore [252,286].

Site-Specific Photocrosslinking (SSPC, biochemical): proteins of interest incorporate a photocrosslinkable amino acid. In vivo interacting proteins are crosslinked with UV-light, and interactions are detected by IP [164].

Nuclear Importing/Redirection Assays (NI, biochemical): A cytoplasmic protein (e.g., due to deletion of the nuclear localization signal) is imported to the nucleus upon interaction with protein imported to the nucleus [232].
**Chromatin Immunoprecipitation** (ChIP, biochemical): Proteins are crosslinked to DNA and precipitated with an antibody. DNA sequence bound can be detected with low- and high-throughput techniques.

**Bimolecular Fluorescence Complementation** (biFC, biochemical): Proteins fused to two split fluorophore fragments can fluoresce upon proximity or interaction [292].

### References

1. Fulton, D.L.; Sundararajan, S.; Badis, G.; Hughes, T.R.; Wasserman, W.W.; Roach, J.C.; Sladek, R. TFCat: The curated catalog of mouse and human transcription factors. *Genome Biol.* 2009, 10, R29. [CrossRef]
2. Lambert, S.A.; Jolma, A.; Campitelli, L.F.; Das, P.K.; Yin, Y.; Albu, M.; Chen, X.; Taipale, J.; Hughes, T.R.; Weirauch, M.T. The Human Transcription Factors. *Cell* 2018, 175, 598–599. [CrossRef] [PubMed]
3. Lemon, B.; Tjian, R. Orchestrated response: A symphony of transcription factors for gene control. *Genes Dev.* 2000, 14, 2551–2569. [CrossRef] [PubMed]
4. Luscombe, N.M.; Austin, S.E.; Berman, H.M.; Thornton, J.M. An overview of the structures of protein-DNA complexes. *Genome Biol.* 2000, 1, 1–37. [CrossRef] [PubMed]
5. Vaquerizas, J.M.; Kummerfeld, S.K.; Teichmann, S.A.; Luscombe, N.M. A census of human transcription factors: Function, expression and evolution. *Nat. Rev. Genet.* 2009, 10, 252–263. [CrossRef]
6. Murre, C. Helix-loop-helix proteins and the advent of cellular diversity: 30 years of discovery. *Genes Dev.* 2019, 33, 6–25. [CrossRef]
7. Murre, C.; Page-McCaw, P.; Vaessen, H.; Caudy, M.; Jan, L.; Jan, Y.N.; Cabrera, C.V.; Buskin, J.N.; Haushka, S.D.; Lassar, A.B.; et al. Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* 1989, 58, 537–544. [CrossRef]
8. Murre, C.; McCaw, P.S.; Baltimore, D. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell* 1989, 56, 777–783. [CrossRef]
9. Davis, R.L.; Cheng, P.-F.; Lassar, A.B.; Weintraub, H. The MyoD DNA binding domain contains a recognition code for muscle-specific gene activation. *Cell* 1990, 60, 733–746. [CrossRef]
10. Voronova, A.; Baltimore, D. Mutations that disrupt DNA binding and dimer formation in the E47 helix-loop-helix protein map to distinct domains. *Proc. Natl. Acad. Sci. USA* 1990, 87, 4722–4726. [CrossRef]
11. Ephrussi, A.; Church, G.M.; Tonegawa, S.; Gilbert, W. B Lineage—Specific Interactions of an Immunoglobulin Enhancer with Cellular Factors in Vivo. *Science* 1985, 227, 134–140. [CrossRef] [PubMed]
12. Ishibashi, M.; Sasai, Y.; Nakashima, S.; Kageyama, R. Molecular characterization of HES-2, a mammalian helix-loop-helix factor structurally related to *Drosophila hairy* and *Enhancer of split*. *Eur. J. Biochem.* 1993, 215, 645–652. [CrossRef] [PubMed]
13. Belanger-Jasmin, S.; Llamosas, E.; Tang, Y.; Joachim, K.; Osiceanu, A.-M.; Jhas, S.; Stifani, S. Inhibition of cortical astrocyte differentiation by Hes6 requires amino- and carboxy-terminal motifs important for dimerization and phosphorylation. *J. Neurochem.* 2007, 103, 2022–2034. [CrossRef] [PubMed]
14. Wang, L.-H.; Baker, N.E. E Proteins and ID Proteins: Helix-Loop-Helix Partners in Development and Disease. *Dev. Cell* 2015, 35, 269–280. [CrossRef]
15. Sun, H.; Ghaffari, S.; Taneja, R. bHLH-Orange Transcription Factors in Development and Cancer. *Transl. Oncogenom.* 2007, 2, 107–120. [CrossRef]
16. Belle, I.; Zhuang, Y. E Proteins in Lymphocyte Development and Lymphoid Diseases. *Curr. Top. Dev. Biol.* 2014, 110, 153–187. [CrossRef]
17. Slattery, C.; Ryan, M.P.; McMorrow, T. E2A proteins: Regulators of cell phenotype in normal physiology and disease. *Int. J. Biochem. Cell Biol.* 2008, 40, 1431–1436. [CrossRef]
18. Barinaga, M. Dimers direct development. *Science* 1991, 251, 1176–1177. [CrossRef]
19. Weintraub, H.; Davis, R.; Tavsscott, S.; Thayer, M.; Krause, M.; Benezra, R.; Blackwell, T.K.; Turner, D.; Rupp, R.; Hollenberg, S.; et al. The myoD Gene Family: Nodal Point during Specification of the Muscle Cell Lineage. *Science* 1991, 251, 761–766. [CrossRef]
20. Benezra, R.; Davis, R.L.; Lockshon, D.; Turner, D.L.; Weintraub, H. The protein Id: A negative regulator of helix-loop-helix DNA binding proteins. *Cell* 1990, 61, 49–59. [CrossRef]
21. Murre, C.; Bain, G.; van Dijk, M.A.; Engel, I.; Furnari, B.A.; Massari, M.E.; Matthews, J.R.; Quong, M.W.; Rivera, R.R.; Stuiver, M.H. Structure and function of helix-loop-helix proteins. *Biochem. Biophys. Acta (BBA)-Gene Struct. Expr.* 1994, 1218, 129–135. [CrossRef]
22. Massari, M.E.; Murre, C. Helix-Loop-Helix Proteins: Regulators of Transcription in Eucaryotic Organisms. *Mol. Cell. Biol.* 2000, 20, 429–440. [CrossRef] [PubMed]
23. Roberts, V.J.; Steenbergen, R.; Murre, C. Localization of E2A mRNA expression in developing and adult rat tissues. *Proc. Natl. Acad. Sci. USA* 1993, 90, 7583–7587. [CrossRef]
24. Zhuang, Y.; Kim, C.G.; Bartelmez, S.; Cheng, P.; Groudine, M.; Weintraub, H. Helix-loop-helix transcription factors E12 and E47 are not essential for skeletal or cardiac myogenesis, erythropoiesis, chondrogenesis, or neurogenesis. *Proc. Natl. Acad. Sci. USA* 1992, 89, 12132–12136. [CrossRef] [PubMed]
25. Ravanpay, A.C.; Olson, J.M. E protein dosage influences brain development more than family member identity. J. Neurosci. Res. 2008, 86, 1472–1481. [CrossRef] [PubMed]

26. Braun, T.; Arnold, H.H. The four human muscle regulatory helix-loop-helix proteins Myf3-Myf6 exhibit similar heterodimerization and DNA binding properties. Nucleic Acids Res. 1991, 19, 5645–5651. [CrossRef]

27. Miyamoto, A.; Cui, X.; Naumovski, L.; Cleary, M.L. Helix-loop-helix proteins LYL1 and E2a form heterodimeric complexes with distinctive DNA-binding properties in hematopoietic cells. Mol. Cell. Biol. 1996, 16, 2394–2401. [CrossRef] [PubMed]

28. Flora, A.; Garcia, J.J.; Thaller, C.; Zoghbi, H.Y. The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. Proc. Natl. Acad. Sci. USA 2007, 104, 15382–15387. [CrossRef]

29. Poulin, G.; Turgeon, B.; Drouin, J. NeuroD1/beta2 contributes to cell-specific transcription of the proopiomelanocortin gene. Mol. Cell. Biol. 1997, 17, 6673–6682. [CrossRef]

30. Murakami, M.; Kataoka, K.; Tominaga, J.; Nakagawa, O.; Kurihara, H. Differential cooperation between dHAND and three different E-proteins. Biochem. Biophys. Res. Commun. 2004, 323, 168–174. [CrossRef]

31. Li, S.; Mattar, P.; Zinyk, D.; Singh, K.; Chaturvedi, C.-P.; Kovach, C.P.; Dixit, R.; Kurrasch, D.M.; Ma, Y.; Chan, J.A.; et al. GSK3 Temporally Regulates Neurogenin 2 Proline Activity in the Neocortex. J. Neurosci. 2012, 32, 7791–7805. [CrossRef]

32. Fischer, B.; Azim, K.; Hurtado-Chong, A.; Ramelli, S.; Fernández, M.; Raineteau, O. E-proteins orchestrate the progression of neural stem cell differentiation in the postnatal forebrain. Neural Dev. 2014, 9, 23. [CrossRef]

33. Pfürr, S.; Chu, Y.-H.; Bohrer, C.; Greulich, F.; Beattie, R.; Mammadzada, K.; Hils, M.; Arnold, S.J.; Taylor, V.; Schachtrup, K.; et al. The E2A splice variant E47 regulates the differentiation of projection neurons via p57(KIP2) during cortical development. Development 2017, 144, 3917–3931. [CrossRef]

34. Bauderlique, T.; Peña-Pérez, L.; Karhari, S.; Hils, M.; Li, X.; Kristic, A.; De Paep, A.; Schachtrup, C.; Gustafsson, C.; Holmberg, D.; et al. The Concerted Action of E2-2 and HEB Is Critical for Early Lymphoid Specification. Front. Immunol. 2019, 10, 455. [CrossRef]

35. Wedel, M.; Fröb, F.; Elsesser, O.; Wittmann, M.-T.; Lie, D.C.; Reis, A.; Wegner, M. Transcription factor Tcf4 is the preferred heterodimerization partner for Olig2 in oligodendrocytes and required for differentiation. Nucleic Acids Res. 2020, 48, 4839–4857. [CrossRef] [PubMed]

36. McLellan, A.S.; Langlands, K.; Kealey, T. Exhaustive identification of human class II basic helix-loop-helix proteins by virtual library screening. Mech. Dev. 2002, 119 (Suppl. 1), S285–S291. [CrossRef]

37. Carroll, P.A.; Freie, B.W.; Mathysara, H.; Eisenman, R.N. The MYC transcription factor network: Balancing metabolism, proliferation and oncogenesis. Front. Mol. 2018, 12, 412–425. [CrossRef] [PubMed]

38. Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. Nucleic Acids Res. 2000, 28, 235–242. [CrossRef]

39. Etzioni, S.; Yafe, A.; Khateb, S.; Weisman-Shomer, P.; Bengal, E.; Fry, M. Homodimeric MyoD Preferentially Binds Tetraplex Structures of Regulatory Sequences of Muscle-specific Genes. J. Biol. Chem. 2005, 280, 26805–26812. [CrossRef]

40. Bersten, D.C.; Sullivan, A.E.; Peet, D.J.; Whitelaw, M.L. bHLH-PAS proteins in cancer. Nat. Rev. Cancer 2013, 13, 827–841. [CrossRef]

41. Kolonko, M.; Greb-Markiewicz, B. bHLH-PAS Proteins: Their Structure and Intrinsic Disorder. Int. J. Mol. Sci. 2019, 20, 3653. [CrossRef] [PubMed]

42. Atchley, W.R.; Fitch, W.M. A natural classification of the basic helix-loop-helix class of transcription factors. Proc. Natl. Acad. Sci. USA 1997, 94, 5172–5176. [CrossRef] [PubMed]

43. Ledent, V.; Vervoort, M. The Basic Helix-Loop-Helix Protein Family: Comparative Genomics and Phylogenetic Analysis. Genome Res. 2001, 11, 754–770. [CrossRef] [PubMed]

44. Ledent, V.; Paquet, O.; Vervoort, M. Phylogenetic analysis of the human basic helix-loop-helix proteins. Genome Biol. 2002, 3. [CrossRef]

45. Crozatier, M.; Valle, D.; Dubois, L.; Ibnsouda, S.; Vincent, A. Collier, a novel regulator of Drosophila head development, is expressed in a single mitotic domain. Curr. Biol. 1996, 6, 707–718. [CrossRef]

46. Jones, S. An overview of the basic helix-loop-helix proteins. Genome Biol. 2004, 5, 226. [CrossRef]

47. Stevens, J.D.; Roalson, E.; Skinner, M.K. Phylex and expression analysis of the basic helix-loop-helix transcription factor gene family: Genomic approach to cellular differentiation. Differentiation 2008, 76, 1006–1042. [CrossRef]

48. Skinner, M.K.; Rawls, A.; Wilson-Rawls, J.; Roalson, E.H. Basic helix-loop-helix transcription factor gene family phylogenetics and nomenclature. Differentiation 2010, 80, 1–8. [CrossRef]

49. Chakraborty, T.; Olson, E.N. Domains outside of the DNA-binding domain impart target gene specificity to myogenin and MRF4. Mol. Cell. Biol. 1991, 11, 6103–6108. [CrossRef]

50. Petropoulos, H.; Skerjanc, I.S. Analysis of the Inhibition of MyoD Activity by ITF-2B and Full-length E12/E47. J. Biol. Chem. 2000, 275, 25095–25101. [CrossRef]

51. Yutzey, K.E.; Rhodes, S.J.; Konieczny, S.F. Differential trans activation associated with the muscle regulatory factors MyoD1, myogenin, and MRF4. Mol. Cell. Biol. 1990, 10, 3934–3944. [PubMed]

52. Quong, M.W.; Massari, M.E.; Zwart, R.; Murre, C. A new transcriptional-activation motif restricted to a class of helix-loop-helix proteins is functionally conserved in both yeast and mammalian cells. Mol. Cell. Biol. 1993, 13, 792–800. [CrossRef]
53. Massari, M.E.; Jennings, P.A.; Murre, C. The AD1 transactivation domain of E2A contains a highly structured helix which is required for its activity in both Saccharomyces cerevisiae and mammalian cells. Mol. Cell Biol. 1996, 16, 121–129. [CrossRef] [PubMed]

54. Lu, J.; Sloan, S.R. The basic helix-loop-helix domain of the E47 transcription factor requires other protein regions for full DNA binding activity. Biochem. Biophys. Res. Commun. 2002, 290, 1521–1528. [CrossRef] [PubMed]

55. Cserjesi, P.; Brown, D.; Ligon, K.L.; Lyons, G.E.; Copeland, N.G.; Gilbert, D.J.; Jenkins, N.A.; Olson, E.N. Scleraxis: A basic helix-loop-helix protein that prefigures skeletal formation during mouse embryogenesis. Development 2012, 1819, 375–381. [CrossRef]

56. Henthorn, P.; Kiledjian, M.; Kadesch, T. Two distinct transcription factors that bind the immunoglobulin enhancer microE5/kappa 2 motif. Science 1990, 247, 467–470. [CrossRef]

57. Pagliuca, A.; Gallo, P.; De Luca, P.; Lania, L. Class A helix-loop-helix proteins are positive regulators of several cyclin-dependent kinase inhibitors’ promoter activity and negatively affect cell growth. Cancer Res. 2000, 60, 1376–1382.

58. Sepp, M.; Kannike, K.; Eesmaa, A.; Urb, M.; Timmusk, T. Functional diversity of human basic helix-loop-helix transcription factor TC4 isoforms generated by alternative S’ exon usage and splicing. PLoS ONE 2001, 6, e22138. [CrossRef] [PubMed]

59. Sobrado, V.R.; Moreno-Bueno, G.; Cubillo, E.; Holt, L.; Nieto, M.A.; Portillo, F.; Cano, A. The class IbHLH factors E2-2A and E2-2B regulate EMT. J. Cell Sci. 2009, 122, 1014–1024. [CrossRef] [PubMed]

60. Furumatsu, T.; Shukunami, C.; Amemiya-Kudo, M.; Shimano, H.; Ozaki, T. Scleraxis and E47 cooperatively regulate the expression of E-box elements. J. Biol. Chem. 2000, 275, 28147–28154. [CrossRef] [PubMed]

61. Hyndman, B.D.; Thompson, P.; Deniz, C.M.; Chitayat, S.; Bayly, R.; Smith, S.P.; LeBrun, D.P. Mapping acetylation sites in E2A interaction with the MyoD family of gene regulatory factors. Mol. Cell Biol. 1991, 16, 1766–1772. [CrossRef] [PubMed]

62. Johnson, S.E.; Wang, X.; Hardy, S.; Taparowsky, E.J.; Konieczny, S.F. Casein kinase II increases the transcriptional activities of MRFP4 and MyoD independently of their direct phosphorylation. Mol. Cell Biol. 1996, 16, 1604–1613. [CrossRef]

63. Yafe, A.; Shkolov, J.; Weissman-Shomer, P.; Bengal, E.; Fry, M. Differential binding of quadruplex structures by MyoD, MRF4 and myogenin. Nucleic Acids Res. 2008, 36, 3916–3925. [CrossRef]

64. Braun, T.; Bober, E.; Winter, B.; Rosenthal, N.; Arnold, H.H. Myf-6, a new member of the human gene family of myogenic determination factors: Evidence for a gene cluster on chromosome 12. EMBO J. 1990, 9, 821–831. [CrossRef]

65. Braun, T.; Buschaussen-Denker, G.; Bober, E.; Tannich, E.; Arnold, H.H. A novel human muscle factor related to but distinct from MyoD1 induces myogenin conversion in 10T1/2 fibroblasts. EMBO J. 1989, 8, 701–709. [CrossRef] [PubMed]

66. Braun, T.; Winter, B.; Bober, E.; Arnold, H.H. Transcriptional activation domain of the muscle-specific gene-regulatory protein myf5. Nature 1990, 346, 663–665. [CrossRef]

67. Brennan, T.J.; Olson, E.N. Myogenin resides in the nucleus and acquires high affinity for a conserved enhancer element on heterodimerization. Genes Dev. 1990, 4, 582–595. [CrossRef] [PubMed]

68. Millar, S.E.; Lader, E.; Liang, L.F.; Dean, J. Oocyte-specific factors bind a conserved upstream sequence required for mouse zona pellucida promoter activity. Mol. Cell Biol. 1991, 11, 6197–6204. [PubMed]

69. Liang, L.; Soyal, S.M.; Dean, J. FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. J. Biol. Chem. 1997, 272, 4939–4947. [CrossRef]

70. Cserjesi, P.; Brown, D.; Ligon, K.L.; Lyons, G.E.; Copeland, N.G.; Gilbert, D.J.; Jenkins, N.A.; Olson, E.N. Scleraxis: A basic helix-loop-helix protein that prefigures skeletal formation during mouse embryogenesis. Development 1995, 121, 1099–1110. [CrossRef] [PubMed]

71. Carlberg, A.L.; Tuan, R.S.; Hall, D.J. Regulation of scleraxis function by interaction with the bHLH protein E47. Mol. Cell Biol. Res. Commun. 2000, 3, 82–86. [CrossRef] [PubMed]
80. Spicer, D.B.; Rhee, J.; Cheung, W.L.; Lassar, A.B. Inhibition of Myogenic bHLH and MEF2 Transcription Factors by the bHLH Protein Twist. Science 1996, 272, 1476–1480. [CrossRef]

81. Hamamori, Y.; Wu, H.Y.; Sartorelli, V.; Kedes, L. The basic domain of myogenic basic helix-loop-helix (bHLH) proteins is the novel target for direct inhibition by another bHLH protein, Twist. Mol. Cell. Biol. 1997, 17, 6563–6573. [CrossRef] [PubMed]

82. Franco, H.L.; Casasnovas, J.; Rodriguez-Medina, J.R.; Cadilla, C.L. Redundant or separate entities?—Roles of Twist1 and Twist2 as molecular switches during gene transcription. Nucleic Acids Res. 2011, 39, 1177–1186. [CrossRef]

83. Gong, X.Q.; Li, L. Dermo-1, a multifunctional basic helix-loop-helix protein, represses MyoD transactivation via the HLH domain, MEF2 interaction, and chromatin deacetylation. J. Biol. Chem. 2002, 277, 12310–12317. [CrossRef]

84. Verzi, M.P.; Anderson, J.P.; Dodou, E.; Kelly, K.K.; Greene, S.B.; North, B.J.; Cripps, R.M.; Black, B.L. N-twist, an evolutionarily conserved bHLH protein expressed in the developing CNS, functions as a transcriptional inhibitor. Dev. Biol. 2002, 249, 174–190. [CrossRef] [PubMed]

85. Morin, S.; Pozzulo, G.; Robitaille, L.; Cross, J.; Nemer, M. MEF2-dependent recruitment of the HAND1 transcription factor results in synergistic activation of target promoters. J. Biol. Chem. 2005, 280, 32272–32278. [CrossRef]

86. Hollenberg, S.M.; Sternglanz, R.; Cheng, P.F.; Weintraub, H. Identification of a new family of tissue-specific basic helix-loop-helix proteins with a two-hybrid system. Mol. Cell Biol. 1995, 15, 3813–3822. [CrossRef]

87. Knoeffler, M.; Meinhardt, G.; Bauer, S.; Loretger, T.; Vasicke, R.; Bloor, D.J.; Kimber, S.J.; Husslein, P. Human Hand1 basic helix-loop-helix (bHLH) protein: Extra-embryonic expression pattern, interaction partners and identification of its transcriptional repressor domains. Biochem. J. 2002, 361, 641–651. [CrossRef] [PubMed]

88. Bounpheng, M.A.; Morrish, T.A.; Dodds, S.G.; Christy, B.A. Negative Regulation of Selected bHLH Proteins by eHAND. Exp. Cell Res. 2000, 257, 320–331. [CrossRef]

89. Scott, I.C.; Anson-Cartwright, L.; Riley, P.; Reda, D.; Cross, J.C. The HAND1 Basic Helix-Loop-Helix Transcription Factor Regulates Trophoblast Differentiation via Multiple Mechanisms. Mol. Cell. Biol. 2000, 20, 530–541. [CrossRef]

90. Firulli, B.A.; Hadzic, D.B.; McDaid, J.R.; Firulli, A.B. The basic helix-loop-helix transcription factors dHAND and eHAND exhibit dimerization characteristics that suggest complex regulation of function. J. Biol. Chem. 2000, 275, 33567–33573. [CrossRef]

91. Dai, Y.S.; Cserjesi, P. The basic helix-loop-helix factor, HAND2, functions as a transcriptional activator by binding to E-boxes as a heterodimer. J. Biol. Chem. 2002, 277, 12604–12612. [CrossRef]

92. Funato, N.; Chapman, S.L.; McKee, M.D.; Funato, H.; Morris, J.A.; Shelton, J.M.; Yanagisawa, H. Hand2 controls osteoblast differentiation in the branchial arch by inhibiting DNA binding of Runx2. Development 2009, 136, 615–625. [CrossRef]

93. Beres, T.M.; Masui, T.; Swift, G.H.; Shi, L.; Henke, R.M.; MacDonald, R.J. PTF1 is an organ-specific and Notch-independent basic helix-loop-helix protein: Redundant or separate entities?—Roles of Twist1 and Twist2 as molecular switches during gene transcription. Nucleic Acids Res. 2011, 39, 1177–1186. [CrossRef]

94. Verzi, M.P.; Anderson, J.P.; Dodou, E.; Kelly, K.K.; Greene, S.B.; North, B.J.; Cripps, R.M.; Black, B.L. N-twist, an evolutionarily conserved bHLH protein expressed in the developing CNS, functions as a transcriptional inhibitor. Dev. Biol. 2002, 249, 174–190. [CrossRef] [PubMed]

95. Mutoh, H.; Fung, B.P.; Naya, F.J.; Tsai, M.J.; Nishitani, J.; Leiter, A.B. The basic helix-loop-helix transcription factor BETA2/NeuroD defines a new transition stage in neurogenesis. Development 2000, 127, 693–702. [CrossRef]

96. Sharma, A.; Moore, M.; Marcara, E.; Lee, J.E.; Qiu, Y.; Samaras, S.; Stein, R. The NeuroD1/BETA2 sequences essential for insulin gene transcription colocalize with those necessary for neurogenesis and p300/CREB binding protein binding. Mol. Cell Biol. 1999, 19, 704–713. [CrossRef]

97. Farah, M.H.; Olson, J.M.; Sucic, H.B.; Hume, R.I.; Tapscott, S.J.; Turner, D.L. Generation of neurons by transient expression of neural bHLH proteins in mammalian cells. Development 2000, 127, 693–702. [CrossRef]

98. Breslin, M.B.; Zhu, M.; Lan, M.S. NeuroD1/E47 regulates the E-box element of a novel zinc finger transcription factor, IA-1, in developing nervous system. J. Biol. Chem. 2003, 278, 38991–38997. [CrossRef] [PubMed]

99. Westernman, B.A.; Chhatta, A.; Poutsma, A.; van Vegchel, T.; Oudejans, C.B. NEUROD1 acts in vitro as an upstream regulator of NEUROD2 in trophoblast cells. Biochim. Biophys. Acta 2004, 1676, 96–103. [CrossRef]

100. Lynn, F.C.; Sanchez, L.; Gomis, R.; German, M.S.; Gasa, R. Identification of the bHLH Factor Math6 as a Novel Component of the Embryonic Pancreas Transcriptional Network. PLoS ONE 2008, 3, e2430. [CrossRef]

101. Sun, Y.; Nadal-Vicens, M.; Misono, S.; Lin, M.; Zubiaga, A.; Hua, X.; Fan, G.; Greenberg, M.E. Neurogenin Promotes Neurogenesis and Inhibits Glial Differentiation by Independent Mechanisms. Cell 2001, 104, 365–376. [CrossRef]

102. Galvez, H.; Tenà, J.J.; Giraldez, F.; Abello, G. The Repression of Atoh1 by Neurogenin1 during Inner Ear Development. Front. Mol. Neurosci. 2017, 10, 321. [CrossRef] [PubMed]
106. Akazawa, C.; Ishibashi, M.; Shimizu, C.; Nakanishi, S.; Kageyama, R. A Mammalian Helix-Loop-Helix Factor Structurally Related to the Product of Drosophila proneural Gene atonal Is a Positive Transcriptional Regulator Expressed in the Developing Nervous System. *J. Biol. Chem.* 1993, 268, 8730–8738. [CrossRef]

107. Henke, R.M.; Meredith, D.M.; Borromeo, M.D.; Savage, T.K.; Johnson, J.E. Ascl1 and Neurop2 form novel complexes and regulate Delta-like3 (Dll3) expression in the neural tube. *Dev. Biol.* 2009, 328, 529–540. [CrossRef]

108. Huang, H.P.; Liu, M.; El-Hodiri, H.B.; Chu, K.; Jamrich, M.; Tsai, M.J. Regulation of the pancreatic islet-specific gene BETA2 (neuroD) by neuronin 3. *Mol. Cell Biol.* 2000, 20, 3292–3307. [CrossRef]

109. Vetere, A.; Li, W.C.; Paroni, F.; Juhl, K.; Guo, L.; Nishimura, W.; Dai, X.; Bonner-Weir, S.; Sharma, A. OVO homologue-like 1 (Ovol1) transcription factor: A novel target of neurogenin-3 in rodent pancreas. *Diabetologia* 2010, 53, 115–122. [CrossRef]

110. Liu, W.; Mo, Z.; Xiang, M. The Ath5 proneural genes function upstream of Brn3 POU domain transcription factor genes to promote retinal ganglion cell development. *Proc. Natl. Acad. Sci. USA* 2001, 98, 1649–1654. [CrossRef]

111. Matter-Sadzinski, L.; Matter, J.M.; Ong, M.T.; Hernandez, J.; Ballivet, M. Specification of neurotransmitter receptor identity in developing retina: The chick ATH5 promoter integrates the positive and negative effects of several bHLH proteins. *Development* 2001, 128, 217–231. [CrossRef] [PubMed]

112. Patel, N.; Varghese, J.; Masaratana, P.; Latunde-Dada, G.O.; Jacob, M.; Simpson, R.J.; McKie, A.T. The transcription factor ATOH8 is regulated by erythropoietic activity and regulates HAMP transcription and cellular pSMAD1,5,8 levels. *Br. J. Haematol.* 2014, 164, 586–596. [CrossRef] [PubMed]

113. Fang, F.; Wasserstein, S.M.; Torres-Vazquez, J.; Feinstein, B.; Cao, F.; Li, Z.; Wilson, K.D.; Yue, W.; Wu, J.C.; Xie, X.; et al. The role of HATH6, a newly identified shear-stress-responsive transcription factor, in endothelial cell differentiation and function. *J. Cell Sci.* 2014, 127, 1428–1440. [CrossRef]

114. Ejarque, M.; Altirriba, J.; Comis, R.; Gasa, R. Characterization of the transcriptional activity of the basic helix–loop–helix (bHLH) transcription factor Athoh. *Biochim. Biophys. Acta (BBA)—Bioenerg.* 2013, 1829, 1175–1183. [CrossRef]

115. Tran, T.; Jia, D.; Sun, Y.; Konieczny, S.F. The bHLH domain of Mist1 is sufficient to activate gene transcription. *Gene Expr.* 2007, 13, 241–253. [CrossRef] [PubMed]

116. Lemercier, C.; To, R.Q.; Carrasco, R.A.; Konieczny, S.F. The basic helix-loop-helix transcription factor Mist1 functions as a transcriptional repressor of myoD. *EMBO J.* 1998, 17, 1412–1422. [CrossRef]

117. Johnson, J.E.; Birren, S.J.; Saito, T.; Anderson, D.J. DNA binding and transcriptional regulatory activity of mammalian achaete-scute homologous (MASH) proteins revealed by interaction with a muscle-specific enhancer. *Proc. Natl. Acad. Sci. USA* 1992, 89, 3596–3600. [CrossRef]

118. Jiang, B.; Kamat, A.; Mendelson, C.R. Hypoxia prevents induction of aromatase expression in human trophoblast cells in culture: Potential inhibitory role of the hypoxia-inducible transcription factor Mash-2 (mammalian achaete-scute homologous protein-2). *Mol. Endocrinol.* 2000, 14, 1661–1673. [CrossRef]

119. Yoshida, S.; Ohko, K.; Takakura, A.; Takebayashi, H.; Okada, T.; Abe, K.; Nabeshima, Y. Sgn1, a basic helix-loop-helix transcription factor delineates the salivary gland duct cell lineage in mice. *Dev. Biol.* 2003, 201, 517–530. [CrossRef]

120. Hsu, H.L.; Wadman, I.; Tsan, J.T.; Baer, R. Positive and negative transcriptional control by the TAL1 helix-loop-helix protein. *Proc. Natl. Acad. Sci. USA* 2001, 98, 1649–1654. [CrossRef]

121. Park, S.T.; Sun, X.H. The Tal1 oncprotein inhibits E47-mediated transcriptional regulation. *Mechanism of inhibition.* *J. Biol. Chem.* 1998, 273, 7030–7037. [CrossRef]

122. Huang, S.; Qiu, Y.; Stein, R.W.; Brandt, S.J. p300 functions as a transcriptional coactivator for the TAL1/SCL oncoprotein. *Oncogene* 1999, 18, 4958–4967. [CrossRef]

123. Huang, S.; Brandt, S.J. mSin3A Regulates Murine Erythroleukemia Cell Differentiation through Association with the TAL1 (or SCL) Transcription Factor. *Mol. Cell. Biol.* 2000, 20, 2248–2259. [CrossRef] [PubMed]

124. Hu, X.; Li, X.; Valverde, K.; Fu, X.; Noguchi, C.; Qiu, Y.; Huang, S. LSD1-mediated epigenetic modification is required for TAL1 function and hematopoiesis. *Proc. Natl. Acad. Sci. USA* 2009, 106, 10141–10146. [CrossRef] [PubMed]

125. Goldfarb, A.N.; Lewandowska, K. Inhibition of cellular differentiation by the SCL/tal oncprotein: Transcriptional repression by an Id-like mechanism. *Blood* 1995, 85, 465–471. [CrossRef]

126. Xia, Y.; Brown, L.; Yang, C.Y.; Tsan, J.T.; Siciliano, M.J.; Espinosa, R., III; Le Beau, M.M.; Baer, R.J. TAL2, a helix-loop-helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia. *Proc. Natl. Acad. Sci. USA* 1991, 88, 11416–11420. [CrossRef] [PubMed]

127. Deleuze, V.; El-Hajj, R.; Challhoub, E.; Dohet, C.; Pinet, V.; Couttet, P.; Mathieu, D. Angiopoietin-2 is a direct transcriptional target of Tal1, Ly11 and LMO2 in endothelial cells. *PLoS ONE* 2012, 7, e40484. [CrossRef]

128. San-Maria, S.; Han, Y.; Suarez Saiz, F.; Trus, M.R.; Minden, M.D. Lyl1 interacts with CREB1 and alters expression of CREB1 target genes. *Biochim. Biophys. Acta* 2008, 1783, 503–517. [CrossRef]

129. Zhong, Y.; Jiang, L.; Hai, H.; Toyokuni, S.; Yamada, Y. Overexpression of a transcription factor LYN1 induces T- and B-cell lymphoma in mice. *Oncogene* 2007, 26, 6937–6947. [CrossRef]

130. Manetopoulos, C.; Hansson, A.; Karlsson, J.; Jonsson, J.I.; Axelsson, H. The LIM-only protein LMO4 modulates the transcriptional activity of HEN1. *Biochem. Biophys. Res. Commun.* 2003, 307, 891–899. [CrossRef]

131. Fox, D.L.; Good, D.J. Nescient helix-loop-helix 2 interacts with signal transducer and activator of transcription 3 to regulate transcription of prohormone convertase 1/3. *Mol. Endocrinol.* 2008, 22, 1438–1448. [CrossRef] [PubMed]
132. Isogai, E.; Ohira, M.; Ozaki, T.; Obi, S.; Nakamura, Y.; Nakagawara, A. Oncogenic LMO3 collaborates with HEN2 to enhance neuroblastoma cell growth through transactivation of Mash1. PLoS ONE 2011, 6, e19297.

133. Massari, M.E.; Rivera, R.R.; Voland, J.R.; Quong, M.W.; Breit, T.M.; van Dongen, J.J.; de Smit, O.; Murre, C. Characterization of ABF-1, a novel basic helix-loop-helix transcription factor expressed in activated B lymphocytes. Mol. Cell Biol. 1998, 18, 3130–3139. [CrossRef] [PubMed]

134. Yang, Z.; MacQuarrie, K.L.; Analau, E.; Tyler, A.E.; Dilworth, F.J.; Cao, Y.; Dieder, S.J.; Tapscott, S.J. MyoD and E-protein heterodimers switch rhombomycosaicoma cells from an arrested myoblast phase to a differentiated state. Genes Dev. 2009, 23, 694–707. [CrossRef]

135. Miyagishi, M.; Hatta, M.; Ohshima, T.; Ishida, J.; Fujii, R.; Nakajima, T.; Fukamizu, A. Cell type-dependent transactivation or repression of mesoderm-restricted basic helix-loop-helix protein, POD-1/Capsulin. Mol. Cell Biochem. 2000, 205, 141–147. [CrossRef]

136. Hidai, H.; Bardales, R.; Goodwin, R.; Quertermous, T.; Quertermous, E.E. Cloning of capsulin, a basic helix-loop-helix factor expressed in progenitor cells of the corona and the coronary arteries. Mech. Dev. 1998, 73, 33–43. [CrossRef]

137. Miyagishi, M.; Hatta, M.; Ohshima, T.; Ishida, J.; Fuji, R.; Nakajima, T.; Fukamizu, A. Cell type-dependent transactivation or repression of mesoderm-restricted basic helix-loop-helix protein, POD-1/Capsulin. Mol. Cell Biochem. 2000, 205, 141–147. [CrossRef]

138. Funato, N.; Ohyama, K.; Kuroda, T.; Nakamura, M. Basic Helix-Loop-Helix Transcription Factor Epicardin/Capsulin/Pod-1 Suppresses Differentiation by Negative Regulation of Transcription. J. Biol. Chem. 2003, 278, 7486–7493. [CrossRef]

139. Franca, M.M.; Ferraz-de-Souza, B.; Santos, M.G.; Lacerio, A.M.; Fragos, M.C.; Latronico, A.C.; Kuick, R.D.; Hammer, G.D.; Lotti, C.F. POD-1 binding to the E-box sequence inhibits SF-1 and StAR expression in human adrenocortical tumor cells. Mol. Cell Endocrinol. 2013, 371, 140–147. [CrossRef]

140. Narumi, O.; Mori, S.; Boku, S.; Tsuji, Y.; Hashimoto, N.; Nishikawa, S.; Yokota, Y. OUT, a novel basic helix-loop-helix transcription factor with an Id-like inhibitory activity. J. Biol. Chem. 2000, 275, 3510–3521. [CrossRef]

141. Malik, S.; Percin, F.E.; Bornholdt, D.; Albrecht, B.; Percesepe, A.; Koch, M.C.; Landi, A.; Fritz, B.; Khan, R.; Muntaz, S.; et al. Mutations affecting the BHLH9A DNA-binding domain cause MSSD, mesosyal synostotic syndactyly with phalangeal reduction, Malik-Percin type. Am. J. Hum. Genet. 2014, 95, 649–659. [CrossRef]

142. Ross, S.E.; McCord, A.E.; Jung, C.; Atan, D.; Mok, S.I.; Hemberg, M.; Kim, T.K.; Salogianissis, J.; Hu, L.; Cohen, S.; et al. Bhlhb5 and Prdm8 form a repressor complex involved in neuronal circuit assembly. Neuron 2012, 73, 292–303. [CrossRef]

143. Peyton, M.; Stellrecht, C.M.; Naya, F.J.; Huang, H.P.; Smith, J.A.; Tsai, M.J. BETA3, a novel helix-loop-helix protein, can act as a negative regulator of BETA2 and MyoD-responsive genes. Mol. Cell Biol. 1996, 16, 626–633. [CrossRef] [PubMed]

144. Xue, Z.P.; Dutra, A.; Stellrecht, C.M.; Wu, C.; Patitorskij, S.J.; Saunders, G.F. Functional and structural characterization of the human gene BHLH85, encoding a basic helix-loop-helix transcription factor. Genomics 2002, 80, 311–318. [CrossRef] [PubMed]

145. Bramblett, D.E.; Copeland, N.G.; Jenkins, N.A.; Tsai, M.J. BHLHB4 is a bHLH transcriptional regulator in pancreas and brain that marks the diencephalic boundary. Genomics 2002, 79, 402–412. [CrossRef]

146. Samanta, J.; Kessler, J.A. Interactions between Id and Olig proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development 2004, 131, 4131–4142. [CrossRef] [PubMed]

147. Silbereis, J.C.; Nobuta, H.; Tsai, H.H.; Heine, V.M.; McKinsey, G.L.; Meijer, D.H.; Howard, M.A.; Petryniak, M.A.; Potter, G.B.; Alberts, J.A.; et al. Olig1 function is required to repress dlx1/2 and interneuron production in Mammalian brain. Neuron 2011, 84, 574–587. [CrossRef]

148. Li, H.; de Faria, J.P.; Andrew, P.; Nitarska, J.; Richardson, W.D. Phosphorylation Regulates OLG2 Cofactor Choice and the Motor Neuron-Oligodendrocyte Fate Switch. Neuron 2011, 69, 918–929. [CrossRef]

149. Novitch, B.G.; Chen, A.I.; Jessell, T.M. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron 2001, 31, 773–789. [CrossRef]

150. Lee, S.K.; Lee, B.; Ruiz, E.C.; Paff, S.L. Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. Genes Dev. 2005, 19, 282–294. [CrossRef]

151. Muller, T.; Anlag, K.; Wildner, H.; Britsch, S.; Treier, M.; Birchmeier, C. The bHLH factor Olig3 coordinates the specification of dorsal neurons in the spinal cord. Genes Dev. 2005, 19, 733–743. [CrossRef]

152. Boudjelal, M.; Taneja, R.; Matsubara, S.; Bouillet, P.; Dolle, P.; Chambon, P. Overexpression of Olig3, a novel retinoic acid-inducible gene of the basic helix-loop-helix family, inhibits mesodermal and promotes neuronal differentiation of P19 cells. Genes Dev. 1997, 11, 2052–2065. [CrossRef]

153. St-Pierre, B.; Flock, G.; Zacksenhaus, E.; Egan, S.E. StrA13 homodimers repress transcription through class B E-box elements. J. Biol. Chem. 2002, 277, 46544–46551. [CrossRef]

154. Azmi, S.; Ozog, A.; Taneja, R. Sharp-1/DEC2 Inhibits Skeletal Muscle Differentiation through Repression of Myogenic Transcription Factors. J. Biol. Chem. 2004, 279, 52643–52652. [CrossRef] [PubMed]

155. Fujimoto, K.; Higuchi, H.; Hashiba, T.; Nakamura, T.; Kawamoto, T.; Sato, F.; Noshiro, M.; Bhawal, U.K.; Sanada, K.; Kato, Y. Transcriptional repression by the basic helix-loop-helix protein Dec2: Multiple mechanisms through E-box elements. Int. J. Mol. Med. 2007, 19, 925–932. [CrossRef] [PubMed]

156. Jen, Y.; Weintraub, H.; Benezra, R. Overexpression of Id protein inhibits the muscle differentiation program: In vivo association of Id with E2A proteins. Genes Dev. 1992, 6, 1466–1479. [CrossRef] [PubMed]
157. Jogi, A.; Persson, P.; Grynfeld, A.; Pahlman, S.; Axelson, H. Modulation of basic helix-loop-helix transcription complex formation by Id proteins during neuronal differentiation. J. Biol. Chem. 2002, 277, 9118–9126. [CrossRef] [PubMed]

158. Loveys, D.A.; Streiff, M.B.; Kato, G.J. E2A basic-helix-loop-helix transcription factors are negatively regulated by serum growth factors and by the Id3 protein. Nucleic Acids Res. 1996, 24, 2813–2820. [CrossRef]

159. Riechmann, V.; van Cruchten, I.; Sablitzky, F. The expression pattern of Id4, a novel dominant negative helix-loop-helix protein, is distinct from Id1, Id2 and Id3. Nucleic Acids Res. 1992, 22, 749–755. [CrossRef]

160. Sun, J.; Kamei, C.N.; Layne, M.D.; Jain, M.K.; Liao, J.K.; Lee, M.E.; Chin, M.T. Regulation of myogenic terminal differentiation by the hairy-related transcription factor CHF2. J. Biol. Chem. 2001, 276, 18591–18596. [CrossRef]

161. Iso, T.; Sartorelli, V.; Poizat, C.; Iezzi, S.; Wu, H.Y.; Chung, G.; Kedes, L.; Hamamori, Y. HERP, a novel heterodimer partner of HES/E(spl) in Notch signaling. Mol. Cell. Biol. 2001, 21, 6080–6089. [CrossRef] [PubMed]

162. Jogi, A.; Persson, P.; Grynfeld, A.; Pahlman, S.; Axelson, H. Modulation of basic helix-loop-helix transcription complex formation by Id proteins during neuronal differentiation. J. Biol. Chem. 2002, 277, 9118–9126. [CrossRef] [PubMed]

163. Lavery, D.N.; Villaronga, M.A.; Walker, M.M.; Patel, A.; Belandia, B.; Bevan, C.L. Repression of androgen receptor activity by HES/E(spl) in Notch signaling. Mol. Cell. Biol. 2001, 21, 6080–6089. [CrossRef] [PubMed]

164. Noguchi, Y.T.; Nakamura, M.; Hino, N.; Nagomi, J.; Tsuji, T.; Sato, T.; Zhang, L.; Tsujikawa, K.; Tanaka, T.; Izawa, K.; et al. Cell-autonomous and redundant roles of Hey1 and HeyL in muscle stem cells: HeyL requires Hey1 to bind diverse DNA sites. Development 2019, 146, dev163618. [CrossRef] [PubMed]

165. Ju, B.G.; Solum, D.; Song, E.J.; Lee, K.J.; Rose, D.W.; Glass, C.K.; Rosenfeld, M.G. Activating the PARP-1 sensor component of the DNA-dependent protein kinase-catalytic subunit. J. Biol. Chem. 2004, 279, 119, 815–829. [CrossRef] [PubMed]

166. Sasai, Y.; Kageyama, R.; Tagawa, Y.; Shimogome, R.; Nakanishi, S. Two mammalian helix-loop-helix factors structurally related to Drosophila hairy and Enhancer of split. Genes Dev. 1996, 6, 2620–2634. [CrossRef]

167. Ross, D.A.; Hannenhalli, S.; Tobias, J.W.; Cooch, N.; Shiekhattar, R.; Kadesch, T. Functional analysis of Hes-1 in preadipocytes. Mol. Endocrinol. 2006, 20, 698–705. [CrossRef]

168. Kawamata, S.; Du, C.; Li, K.; Lavau, C. Overexpression of the Notch target genes Hes in vivo induces lymphoid and myeloid alterations. Oncogene 2002, 21, 3853–3863. [CrossRef] [PubMed]

169. Murato, Y.; Yamaguti, M.; Katamura, M.; Cho, K.W.; Hashimoto, C. Two modes of action by which Xenopus hairy2b establishes tissue demarcation in the Spemann-Mangold organizer. Int. J. Dev. Biol. 2002, 46, 471–479. [CrossRef]

170. Akazawa, C.; Sasai, Y.; Nakanishi, S.; Kageyama, R. Molecular characterization of a rat negative regulator with a basic helix-loop-helix structure predominantly expressed in the developing nervous system. J. Biol. Chem. 1992, 267, 21879–21885. [CrossRef]

171. Gao, X.; Chandra, T.; Gratton, M.O.; Quelo, I.; Prud’homme, J.; Stifani, S.; St-Arnaud, R. HES6 acts as a transcriptional repressor in myoblasts and can induce the myogenic differentiation program. J. Cell. Biol. 2001, 154, 1161–1171. [CrossRef] [PubMed]

172. Cossins, J.; Vernon, A.E.; Zhang, Y.; Philpott, A.; Jones, P.H. Hes6 regulates myogenic differentiation. Development 2002, 129, 2195–2207. [CrossRef] [PubMed]

173. Bae, S.; Bessho, Y.; Hojo, M.; Kageyama, R. The bHLH gene Hes6, an inhibitor of Hes1, promotes neuronal differentiation. Development 2000, 127, 2933–2943. [CrossRef] [PubMed]

174. Gratton, M.O.; Torban, E.; Jasmin, S.B.; Theriault, F.M.; German, M.S.; Stifani, S. Hes6 promotes cortical neurogenesis and inhibits Hes1 transcription repression activity by multiple mechanisms. Mol. Cell Biol. 2003, 23, 6922–6935. [CrossRef] [PubMed]

175. Bessho, Y.; Miyoshi, G.; Sakata, R.; Kageyama, R. Hes7: A bHLH-type repressor gene regulated by Notch and expressed in the presomitic mesoderm. Genes Cells 2001, 6, 175–185. [CrossRef] [PubMed]

176. Nakatani, T.; Mizuhashi, E.; Minakawa, Y.; Ono, Y.; Helt, a novel basic-helix-loop-helix transcriptional repressor expressed in the developing central nervous system. J. Biol. Chem. 2004, 279, 16356–16367. [CrossRef]

177. Ryan, D.P.; Duncan, J.L.; Lee, C.; Kuchel, P.W.; Matthews, J.M. Assembly of the oncogenic DNA-binding complex LMO2-Ldb1-TAL1-E12. Proteins: Struct. Funct. Bioinform. 2007, 70, 1461–1474. [CrossRef]

178. Fairman, R.; Beran-Steed, R.K.; Anthony-Cahill, S.J.; Lear, J.D.; Stafford, W.F., III; DeGrado, W.F.; Benfield, P.A.; Brenner, S.L. Multiple oligomeric states regulate the DNA binding of helix-loop-helix peptides. Proc. Natl. Acad. Sci. USA 1993, 90, 10429–10433. [CrossRef]

179. Jolma, A.; Yin, Y.; Nitta, K.; Dave, K.; Popov, A.; Taipale, M.; Enge, M.; Kivioja, T.; Morgunova, E.; Taipale, J. DNA-dependent formation of transcription factor pairs alters their binding specificity. Nature 2015, 527, 384–388. [CrossRef]

180. Slattery, M.; Riley, T.; Liu, P.; Abe, N.; Gomez-Alcala, P.; Dror, I.; Zhou, T.; Rohs, R.; Honig, B.; Bussmacker, H.J.; et al. Cofactor Binding Evokes Latent Differences in DNA Binding Specificity between Hox Proteins. Cell 2011, 147, 1270–1282. [CrossRef]

181. El Omari, K.; Hoosally, S.J.; Taladhar, K.; Karia, D.; Hall-Ponselé, E.; Platonova, O.; Vyas, P.; Patient, R.; Porcher, C.; Mancini, E.J. Structural Basis for LMO2-Driven Recruitment of the SCL:E47bHLH Heterodimer to Hematopoietic-Specific Transcriptional Targets. Cell Rep. 2013, 4, 135–147. [CrossRef] [PubMed]

182. Matthews, J.M.; Lester, K.; Joseph, S.; Curtis, D.J. LIM-domain-only proteins in cancer. Nat. Rev. Cancer 2013, 13, 111–122. [CrossRef] [PubMed]
183. Langlands, K.; Yin, X.; Anand, G.; Prochownik, E.V. Differential Interactions of Id Proteins with Basic-Helix-Loop-Helix Transcription Factors. *J. Biol. Chem.* 1997, 272, 19785–19793. [CrossRef]

184. Li, L.; Cserjesi, P.; Olson, E.N. Dermo-1: A novel twist-related bHLH protein expressed in the developing dermis. *Dev. Biol.* 1995, 172, 280–292. [CrossRef]

185. Spinner, D.S.; Liu, S.; Wang, S.W.; Schmidt, J. Interaction of the myogenic determination factor myogenin with E12 and a DNA target: Mechanism and kinetics. *J. Mol. Biol.* 2002, 317, 431–445. [CrossRef] [PubMed]

186. Takahashi, Y.; Takagi, A.; Hiraoka, S.; Koseki, H.; Kanno, J.; Rawls, A.; Saga, Y. Transcription factors Mesp2 and Paraxis have critical roles in axial musculoskeletal formation. *Dev. Dyn.* 2007, 236, 1484–1494. [CrossRef]

187. Zhao, H.; Chen, Z.J.; Qin, Y.; Shi, Y.; Wang, S.; Choi, Y.; Simpson, J.L.; Rajkovic, A. Transcription factor FIGLA is mutated in patients with premature ovarian failure. *Am. J. Hum. Genet.* 2008, 82, 1342–1348. [CrossRef] [PubMed]

188. Dear, T.N.; Hainzl, T.; Follo, M.; Nehls, M.; Wilmore, H.; Matena, K.; Boehm, T. Identification of interaction partners for the basic-helix-loop-helix protein E47. *Oncogene* 1997, 14, 891–898. [CrossRef] [PubMed]

189. Muir, T.; Sadler-Riggleman, I.; Skinner, M.K. Role of the basic helix-loop-helix transcription factor, scleraxis, in the regulation of Sertoli cell function and differentiation. *Mol. Endocrinol.* 2005, 19, 2164–2174. [CrossRef] [PubMed]

190. Muir, T.; Wilson-Rawls, J.; Stevens, J.D.; Rawls, A.; Schweitzer, R.; Kang, C.; Skinner, M.K. Integration of CREB and bHLH transcriptional signaling pathways through direct heterodimerization of the proteins: Role in muscle and testis development. *Mol. Reprod. Dev.* 2008, 75, 1637–1652. [CrossRef]

191. Blanar, M.A.; Crossley, P.H.; Peters, K.G.; Steinrigger, E.; Copeland, N.G.; Jenkins, N.A.; Martin, G.R.; Rutter, W.J. Meso1, a basic-helix-loop-helix protein involved in mammalian presomitic mesoderm development. *Proc. Natl. Acad. Sci. USA* 1995, 92, 5870–5874. [CrossRef]

192. Palumbo-Zerr, K.; Soare, A.; Zerr, P.; Liebl, A.; Mancuso, R.; Tomcik, M.; Sumova, B.; Dees, C.; Chen, C.-W.; Wohlfahrt, T.; et al. Composition of TWIST1 dimers regulates fibroblast activation and tissue fibrosis. *Ann. Rheum. Dis.* 2016, 76, 244–251. [CrossRef]

193. Firulli, B.A.; Redick, B.A.; Conway, S.J.; Firulli, A.B. Altered Twist1 and Hand2 dimerization is associated with Saethre-Chotzen syndrome and limb abnormalities. *Nat. Genet.* 2005, 37, 373–381. [CrossRef] [PubMed]

194. Teacheron, R.; Beck, K.; Wright, L.Y.T.; Shen, Z.; Briggs, S.P.; Murre, C. Biochemical and Phosphoproteomic Analysis of the Helix-Loop-Helix Protein E47. *Mol. Cell. Biol.* 2012, 32, 1671–1682. [CrossRef] [PubMed]

195. Kophengnadvong, T.; Michnowicz, J.E.; Blackwell, T.K. Establishment of distinct MyoD, E2A, and twist DNA binding specificities by different basic-region-DNA conformations. *Mol. Cell Biol.* 2000, 20, 261–272. [CrossRef] [PubMed]

196. Connerney, J.; Andreeva, V.; Leshem, Y.; Muentener, C.; Mercado, M.A.; Spicer, D.B. Twist1 dimer selection regulates cranial suture patterning and fusion. *Dev. Dyn.* 2006, 235, 1334–1346. [CrossRef] [PubMed]

197. Birrell, B.A.; Redick, B.A.; Conway, S.J.; Firulli, A.B. Mutations within Helix I of Twist1 Result in Distinct Limb Defects and Variation of DNA Binding Affinities. *J. Biol. Chem.* 2007, 282, 27536–27546. [CrossRef] [PubMed]

198. Firulli, B.A.; Howard, M.J.; McDaid, J.R.; McIlreavey, L.; Dionne, K.M.; Centonze, V.E.; Cserjesi, P.; Laufer, E.; Firulli, A.B. Altered Twist1 and Hand2 dimerization is associated with Saethre-Chotzen syndrome and limb abnormalities. *Nat. Genet.* 2003, 37, 373–381. [CrossRef] [PubMed]

199. Hill, A.A.; Riley, P.R. Differential regulation of Hand1 homodimer and Hand1-E12 heterodimer activity by the cofactor FHL2. *Mol. Cell. Biol.* 2004, 24, 9835–9847. [CrossRef] [PubMed]

200. Topno, N.S.; Kannan, M.; Krishna, R. Mechanistic insights into the activity of Ptf1-p48 (pancreas transcription factor 1a): Probing the interactions levels of Ptf1-p48 with E2A-E47 (transcription factor E2-alpha) and ID3 (inhibitor of DNA binding 3). *J. Biol. Chem.* 2012, 287, 19785–19793. [CrossRef]

201. Meredith, D.M.; Masui, T.; Swift, G.H.; Macdonald, R.J.; Johnson, J.E. Multiple Transcriptional Mechanisms Control Ptf1a Levels during Neural Development Including Autoregulation by the PFT1-J Complex. *J. Neurosci.* 2009, 29, 11139–11148. [CrossRef] [PubMed]

202. Kim, J.Y.; Chu, K.; Kim, H.J.; Seong, H.A.; Park, K.C.; Sanyal, S.; Takeda, J.; Ha, H.; Shong, M.; Tsai, M.J.; et al. Orphan nuclear receptor small heterodimer partner, a novel corepressor for a basic helix-loop-helix transcription factor BET2/neuroD. *Mol. Endocrinol.* 2004, 18, 776–790. [CrossRef] [PubMed]

203. Longo, A.; Guanga, G.P.; Rose, R.B. Crystal structure of E47-NeuroD1/beta2 bHLH domain-DNA complex: Heterodimer selectivity and DNA recognition. *Biochemistry* 2008, 47, 218–229. [CrossRef] [PubMed]

204. Mehmood, R.; Yasuhara, N.; Oe, S.; Nagai, M.; Yoneda, Y. Synergistic nuclear import of NeuroD1 and its partner transcription factor, E47, via heterodimerization. *Exp. Cell Res.* 2009, 315, 1639–1652. [CrossRef]

205. Ray, S.K.; Leiter, A.B. The Basic Helix-Loop-Helix Transcription Factor NeuroD1 Facilitates Interaction of Sp1 with the Secretin Gene Enhancer. *Mol. Cell. Biol.* 2007, 27, 7839–7847. [CrossRef]

206. Gradwohl, G.; Fode, C.; Guillemot, F. Restricted expression of a novel murine atonal-related bHLH protein in undifferentiated neural precursors. *Dev. Biol.* 1996, 180, 227–241. [CrossRef]

207. Qiu, Y.; Guo, M.; Huang, S.; Stein, R. Acetylation of the BET2 Transcription Factor by p300-associated Factor Is Important in Insulin Gene Expression. *J. Biol. Chem.* 2004, 279, 9796–9802. [CrossRef]

208. Lee, S.-K.; Pfaff, S.L. Synchronization of Neurogenesis and Motor Neuron Specification by Direct Coupling of bHLH and Homeodomain Transcription Factors. *Neuron* 2003, 38, 731–745. [CrossRef]
209. Shimizu, C.; Akazawa, C.; Nakanishi, S.; Kageyama, R. MATH-2, a mammalian helix-loop-helix factor structurally related to the product of Drosophila proneural gene, is specifically expressed in the nervous system. *Eur. J. Biochem.* 1995, 229, 239–248.  

210. Cheng, Y.-F.; Tong, M.; Edge, A.S.B. Destabilization of Atoh1 by E3 Ubiquitin Ligase Huw1 and Casein Kinase 1 Is Essential for Normal Sensory Hair Cell Development. *J. Biol. Chem.* 2016, 291, 21096–21109. [CrossRef]  

211. Roark, R.; Itzhaki, L.; Philpott, A. Complex regulation controls Neurogenin3 proteolysis. *Biol. Open* 2012, 1, 1264–1272. [CrossRef]  

212. Atac, D.G.; Koller, S.; Hanson, J.V.M.; Feil, S.; Tiwari, A.; Bahr, A.; Baehr, L.; Magyar, I.; Kottke, R.; Gerth-Kahler, C.; et al. Atonal homolog 7 (AtoH7) loss-of-function mutations in predominant bilateral optic nerve hypoplasia. *Hum. Mol. Genet.* 2019, 29, 132–148. [CrossRef] [PubMed]  

213. Lemercier, C.; To, R.Q.; Swansonbc, B.J.; Lyons, G.E.; Konieczny, S.F. Mist1: A Novel Basic Helix-Loop-Helix Transcription Factor Exhibits a Developmentally Regulated Expression Pattern. *Dev. Biol.* 1997, 182, 101–113. [CrossRef] [PubMed]  

214. Meierhans, D.; El-Ariss, C.; Neuenschwander, M.; Sieber, M.; Stackhouse, J.F.; Allemann, R.K. DNA Binding Specificity of the Basic-Helix-Loop-Helix Protein MASH-1. *Biochemistry* 1995, 34, 11026–11036. [CrossRef] [PubMed]  

215. Srisuranpong, V.; Borges, M.W.; Strock, C.L.; Nakakura, E.K.; Watkins, D.N.; Blaumueller, C.M.; Nelson, B.D.; Ball, D.W. Notch Signaling Induces Rapid Degradation of Achaete-Scute Homolog. *Mol. Cell. Biol.* 2002, 22, 3129–3139. [CrossRef]  

216. Hsu, H.L.; Cheng, J.T.; Chen, Q.; Baer, R. Enhancer-binding activity of the tal-1 oncprotein in association with the E47/E12 helix-loop-helix proteins. *Mol. Cell Biol.* 1991, 11, 3037–3042.  

217. Xia, Y.; Hwang, L.Y.; Cobb, M.; Baer, R. Products of the TAL2 oncoprotein in leukemia T cells: bHLH phosphoproteins with DNA-binding activity. *Oncogene* 1994, 9, 1437–1446. [PubMed]  

218. Brown, L.; Baer, R. HEN1 encodes a 20-kilodalton phosphoprotein that binds an extended E-box motif as a homodimer. *Mol. Cell Biol.* 1994, 14, 1245–1255.  

219. Lu, J.; Richardson, J.A.; Olson, E.N. Capsulin: A novel bHLH transcription factor expressed in epicardial progenitors and mesenchyme of visceral organs. *Dev. Mech.* 1998, 73, 23–32. [CrossRef]  

220. Miyagishi, M.; Nakajima, T.; Fukamizu, A. Molecular characterization of mesoderm-restricted basic helix-loop-helix protein, POD-1/Capsulin. *Int. J. Mol. Med.* 2000, 5, 27–31. [CrossRef]  

221. Azmi, S.; Sun, H.; Ozog, A.; Taneja, R. mSharp-1/DEC2, a Basic Helix-Loop-Helix Protein Functions as a Transcriptional Repressor and Translocates to the Nucleus upon Binding DNA. *Mech. Dev.* 2004, 121, 239–248. [CrossRef]  

222. Amselh, D.; El-Ariss, C.; Neuenschwander, M.; Sieber, M.; Stackhouse, J.F.; Allemann, R.K. DNA Binding Specificity of the Basic-Helix-Loop-Helix Protein MASH-1. *Biochemistry* 1995, 34, 11026–11036. [CrossRef] [PubMed]  

223. Li, X.; Wang, W.; Wang, J.; Malovannaya, A.; Xi, Y.; Li, W.; Guerra, R.; Hawke, D.H.; Qin, J.; Chen, J. Proteomic analyses reveal distinct chromatin-associated and soluble transcription factor complexes. *Mol. Syst. Biol.* 2015, 11, 775. [CrossRef] [PubMed]  

224. Kotlyar, M.; Pastrello, C.; Pivetta, F.; Sardo, A.L.; Cumbaa, C.; Li, H.; Naranian, T.; Niu, Y.; Ding, Z.; Vafaee, F.; et al. In silico prediction of physical protein interactions and characterization of interactome orphans. *Nat. Methods* 2014, 12, 79–84. [CrossRef] [PubMed]  

225. Sepp, M.; Pruunsild, P.; Timmusk, T. Pitt–Hopkins syndrome-associated mutations in TCF4 lead to variable impairment of transcription factor function ranging from hypomorphic to dominant-negative effects. *Hum. Mol. Genet.* 2012, 21, 2873–2888. [CrossRef] [PubMed]  

226. Hsu, H.L.; Huang, L.; Tsan, J.T.; Funk, W.; Wright, W.E.; Hu, J.S.; Kingston, R.E.; Baer, R. Preferred sequences for DNA recognition by the TAL1 helix-loop-helix proteins. *Cell Biol. Cell. Biol.* 1994, 14, 1256–1265.  

227. Huttlin, E.L.; Bruckner, R.J.; Paulo, J.A.; Cannon, J.R.; Ting, L.; Baltier, K.; Colby, G.; Gebreab, F.; Gygi, M.P.; Parzen, H.; et al. Architecture of the human interactome defines protein communities and disease networks. *Nature* 2017, 545, 505–509. [CrossRef]  

228. Sun, X.H.; Baltimore, D. An inhibitory domain of E12 transcription factor prevents DNA binding in E12 homodimers but not in E12 heterodimers. *Cell* 1991, 64, 459–470. [CrossRef]  

229. Neuhold, L.A.; Wold, B. HLH forced dimers: Tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. *Cell* 1993, 74, 1033–1042. [CrossRef]  

230. Thayer, M.J.; Weintraub, H. A cellular factor stimulates the DNA-binding activity of MyoD and E47. *Proc. Natl. Acad. Sci. USA* 1993, 90, 6483–6487. [CrossRef]  

231. Blackwell, T.K.; Weintraub, H. Differences and similarities in DNA-binding preferences of MyoD and E2A protein complexes revealed by binding site selection. *Science* 1990, 250, 1104–1110. [CrossRef]  

232. Wendt, H.; Thomas, R.M.; Ellenberger, T. DNA-mediated folding and assembly of MyoD-E47 heterodimers. *J. Biol. Chem.* 1998, 273, 5735–5743. [CrossRef] [PubMed]  

233. Lingbeck, J.M.; Trausch-Azar, J.S.; Ciechanover, A.; Schwartz, A.L. E12 and E47 modulate cellular localization and proteasome-mediated degradation of MyoD and Id1. *Oncogene* 2005, 24, 6376–6384. [CrossRef] [PubMed]  

234. Molkentin, J.D.; Black, B.L.; Martin, J.F.; Olson, E.N. Cooperative activation of muscle gene expression by MEF2 and myogenic bHLH proteins. *Cell* 1995, 83, 1125–1136. [CrossRef]  

235. Hsu, H.L.; Wadman, I.; Baer, R. Formation of in vivo complexes between the TAL1 and E2A polypeptides of leukemic T cells. *Proc. Natl. Acad. Sci. USA* 1994, 91, 3181–3185. [CrossRef] [PubMed]  

236. Wadman, I.; Li, J.; Bash, R.; Forster, A.; Osada, H.; Rabbitts, T.; Baer, R. Specific in vivo association between the bHLH and LIM proteins implicated in human T cell leukemia. *EMBO J.* 1994, 13, 4831–4839. [CrossRef]  

237. Fu, J.; Qin, L.; He, T.; Qin, J.; Hong, J.; Wong, J.; Liao, L.; Xu, J. The TWIST/Mi2/NuRD protein complex and its essential role in cancer metastasis. *Cell Res.* 2011, 21, 275–289. [CrossRef]
237. Sato, F.; Kawamoto, T.; Fujimoto, K.; Noshiro, M.; Honda, K.K.; Honna, S.; Honma, K.; Kato, Y. Functional analysis of the basic helix-loop-helix transcription factor DEC1 in circadian regulation. Interaction with BMAL1. *Eur. J. Biochem.* 2004, 271, 4409–4419. [CrossRef]

238. Teo, Z.; Chan, J.S.K.; Chong, H.C.; Sng, M.K.; Choo, C.C.; Phua, G.Z.M.; Teo, D.J.R.; Zhu, P.; Choong, C.; Wong, M.T.C.; et al. Angiopoietin-like 4 induces a beta-catenin-mediated upregulation of ID3 in fibroblasts to reduce scar collagen expression. *Sci. Rep.* 2017, 7, 6303. [CrossRef] [PubMed]

239. Cakouros, D.; Isenmann, S.; Hemming, S.E.; Menicanin, D.; Camp, E.; Zannetinno, A.C.W.; Gronthos, S.; Zannettino, A.C.W. Novel Basic Helix–Loop–Helix Transcription Factor Hes4 Antagonizes the Function of Twist-1 to Regulate Lineage Commitment of Bone Marrow Stromal/Stem Cells. *Stem Cells Dev.* 2015, 24, 1297–1308. [CrossRef]

240. Ghosh, B.; Leach, S.D. Interactions between Hairy/Enhancer of Split-related proteins and the pancreatic transcription factor Ptf1-p48 modulate function of the PTF1 transcriptional complex. *Biochem. J.* 2006, 393, 679–685. [CrossRef] [PubMed]

241. Taelman, V.; Van Weyerbergh, R.; Solter, M.; Pichon, B.; Piefer, T.; Christophe, D.; Bellefroid, E.J. Sequences downstream of the bHLH domain of the Xenopus hairy-related transcription factor 1-act as an extended dimerization domain that contributes to the selection of the partners. *Dev. Biol.* 2004, 276, 47–63. [CrossRef]

242. Solter, M.; Locker, M.; Boy, S.; Taelman, V.; Bellefroid, E.J.; Perron, M.; Piefer, T. Characterization and function of the bHLH-O protein XHes2: Insight into the mechanisms controlling retinal cell fate decision. *Development* 2006, 133, 4097–4108. [CrossRef]

243. Liu, A.; Li, J.; Marin-Husstege, M.; Kageyama, R.; Fan, Y.; Gelinas, C.; Casaccia-Bonnefil, P. A molecular insight of Hes5-dependent inhibition of myelin expression: Old partners and new players. *EMBO J.* 2006, 25, 4833–4842. [CrossRef] [PubMed]

244. Wende, C.-Z.; Zoubaa, S.; Blak, A.; Echevarria, D.; Martinez, S.; Guillemot, F.; Wurst, W.; Guimera, J. Hairy/Enhancer-of-Split MEGANe and Proneural MASH1 Factors Cooperate Synergistically in Midbrain GABAergic Neurogenesis. *PLoS ONE* 2015, 10, e0127681. [CrossRef] [PubMed]

245. Sotoca, A.M.; Prange, K.H.; Rejinders, B.; Mandoli, A.; Nguyen, L.N.; Stunnenberg, H.G.; Martens, J.H. The oncofusion protein FUS–ERG targets key hematopoietic regulators and modulates the all-trans retinoic acid signaling pathway in t(16;21) acute myeloid leukemia. *Oncogene* 2015, 34, 1965–1976. [CrossRef]

246. Springhorn, J.P.; Singh, K.; Kelly, R.A.; Smith, T.W. Posttranscriptional regulation of Id1 activity in cardiac muscle. Alternative splicing of novel Id1 transcript permits homodimerization. *J. Biol. Chem.* 1994, 269, 5132–5136. [CrossRef]

247. Guo, S.-J.; Hu, J.-G.; Zhao, B.-M.; Shen, L.; Wang, R.; Zhou, J.-S.; Lu, H.-Z. Olig1 and ID4 interactions in living cells visualized by bimolecular fluorescence complementation technique. *Mol. Biol. Rep.* 2010, 38, 4637–4642. [CrossRef]

248. Ellenberger, T.; Fass, D.; Arnaud, M.; Harrison, S.C. Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer. *Genes Dev.* 1994, 8, 970–980. [CrossRef] [PubMed]

249. Yang, J.; Horton, J.R.; Li, J.; Huang, Y.; Zhang, X.; Blumenthal, R.M.; Cheng, X. Structural basis for preferential binding of human TCF4 to DNA containing 5-carboxylcytosine. *Nucleic Acids Res.* 2019, 47, 8375–8387. [CrossRef]

250. Ma, P.C.; Rould, M.A.; Weintraub, H.; Pabo, C.O. Crystal structure of MyoD bHLH domain-DNA complex: Perspectives on DNA recognition and implications for transcriptional activation. *Cell* 1994, 77, 451–459. [CrossRef]

251. Liu, Y.; Watanabe, H.; Nifujii, A.; Yamada, Y.; Olson, E.N.; Noda, M. Overexpression of a single helix-loop-helix-type transcription factor, scleraxis, enhances aggrecan gene expression in osteoblastic osteosarcoma ROS17/2.8 cells. *J. Biol. Chem.* 1997, 272, 29880–29885. [CrossRef]

252. Centonze, V.E.; Firulli, B.A.; Firulli, A.B. Fluorescence resonance energy transfer (FRET) as a method to calculate the dimerization strength of basic Helix-Loop-Helix (bHLH) proteins. *Biochem. Proced. Online* 2004, 6, 78–82. [CrossRef]

253. Aguado-Llera, D.; Goormaghtigh, E.; de Geest, N.; Quan, X.J.; Prieto, A.; Hassan, B.A.; Gomez, J.; Neira, J.L. The basic helix-loop-loop region helix-loop-helix transcription factor dimerization. *Biochemistry* 2004, 43, 970–980. [CrossRef] [PubMed]

254. Zhu, L.; Tran, T.; Rukstalis, J.M.; Sun, P.; Damsz, B.; Konieczny, S.F. Inhibition of Mist1 homodimer formation induces pancreatic acinar-to-duodenal metaplasia. *Mol. Cell. Biol.* 2004, 24, 2673–2681. [CrossRef] [PubMed]

255. Künne, A.G.E.; Sieber, M.; Meierhans, A.D.; Allemann, R.K. Thermodynamics of the DNA Binding Reaction of Transcription Factor MASH-1. *Biochemistry* 1998, 37, 4217–4223. [CrossRef]

256. Oasa, S.; Vukojevic, V.; Rigler, R.; Tsigeln, I.F.; Changeux, J.-P.; Terenius, L. A strategy for designing allosteric modulators of transcription factor dimerization. *Proc. Natl. Acad. Sci. USA* 2020, 117, 2683–2686. [CrossRef] [PubMed]

257. Zhou, Q.; Choi, G.; Anderson, D.J. The bHLH Transcription Factor Olig2 Promotes Oligodendrocyte Differentiation in Collaboration with Nkx2. *Neuron* 2001, 31, 791–807. [CrossRef]

258. Wright, W.E.; Binder, M.; Funk, W. Cyclic amplification and selection of targets (CASTing) for the myogenin consensus binding site. *Mol. Cell. Biol.* 1991, 11, 4104–4110. [CrossRef]

259. Xing, S.; Wallmorth, N.; Berendzen, K.; Grefen, C. Techniques for the analysis of protein-protein interactions in vivo. *Plant Physiol.* 2016, 171, 727–758. [CrossRef]

260. Podobnik, M.; Krasevec, N.; Bedina Zavec, A.; Naneh, O.; Flasker, A.; Caserman, S.; Hodnik, V.; Anderluh, G. How to Study Protein-protein Interactions. *Acta Chim. Slov.* 2016, 63, 424–439. [CrossRef]

261. Orchard, S.; Ammari, M.; Aranda, B.; Breuza, L.; Briganti, L.; Broackes-Carter, F.; Campbell, N.H.; Chavali, G.; Chelli, C.; del-Toro, N.; et al. The MIntAct project—IntAct as a common curation platform for 11 molecular interaction databases. *Nucleic Acids Res.* 2014, 42, D358–D363. [CrossRef]
291. Stynen, B.; Tournu, H.; Tavernier, J.; Van Dijck, P. Diversity in Genetic In Vivo Methods for Protein-Protein Interaction Studies: From the Yeast Two-Hybrid System to the Mammalian Split-Luciferase System. *Microbiol. Mol. Biol. Rev.* 2012, 76, 331–382. [CrossRef]

292. Hu, C.-D.; Chinenov, Y.; Kerppola, T.K. Visualization of Interactions among bZIP and Rel Family Proteins in Living Cells Using Bimolecular Fluorescence Complementation. *Mol. Cell* 2002, 9, 789–798. [CrossRef]